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Catecholamine Action on Smooth Muscle

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I. Introduction

EVERY organ contains smooth muscles. Their functions and properties differ depending on the organ in which they occur and on the particular location within the organ. The activity of smooth muscle can be influenced by many factors, but it is mainly regulated through the autonomic nervous system and through hormones in the circulation. Both norepinephrine, the sympathetic neurotransmitter, and epinephrine, the hormone released from the adrenal medulla, have strong effects on smooth muscle activity. Their action is very complex and, although there are recent significant advances in our understanding, many unsolved problems remain concerning the nature of different receptors and, most important, the mechanisms by which the catecholamines produce their effects.

The inherent properties of smooth muscles are generally correlated with the electrical activity of the plasma membrane, as pointed out by Creed (92). The pattern of the electrical activity varies greatly, and different smooth muscles can therefore be classified according to their excitability, although a clear definition of distinct categories is often difficult. Some muscles, such as large blood vessels, do not produce a clear active response, while others, such as the taenia of guinea pig caecum, produce action potentials of a simple spike type in an all-or-nothing manner. However, most smooth muscles have intermediate properties; i.e., the evoked action potential is graded in amplitude, depending on stimulus intensity, and the configuration of the action potential is variable. Poorly excitable muscles are inevitably quiescent and generally lack muscle tone, but highly excitable muscles may exhibit spontaneous myogenic activity, and the frequency of spike discharge determines the pattern of mechanical activity. Quiescent, but excitable muscles respond to nerve stimulation with depolarization (the excitatory junction potential) which leads to the generation of action potentials. These differences in electrical properties might be expected to be correlated with the different mechanisms by which catecholamines affect the activity of different muscles.

This review is confined to the action of catecholamines on postjunctional receptors located in the smooth muscle cell membrane, as observed, in most experiments, on isolated preparations. The review has been restricted mainly to those observations in which the mechanism of the catecholamine action has been analysed to some extent. The material has been arranged, as far as possible, according to the order of excitability of the muscles that have been investigated. One would naturally expect that the inhibitory control is dominant in a muscle with high spontaneous activity, whereas the excitatory control would be dominant in a quiescent muscle. There is such a tendency, but our aim, to find a possible correlation between the mode of action of catecholamines and the properties of smooth muscle, has only partially been achieved, because of the lack of necessary information.

One has an impression that, the more the studies are expanded, the greater the confusion. This may be an inevitable result of the real diversity of smooth muscles in different organs and different species. On the other hand, the confusion may have come from the interpretation of results obtained without appropriate analysis of the individual electrical and mechanical properties of the smooth muscle under investigation. Though this review may, therefore, be unable to present clear-cut conclusions, it is hoped that it may be useful in indicating possible approaches for future research.

II. Distribution of Adrenoceptors

Adrenergic receptors have been classified into α- and β-receptors (8). The β-adrenoceptors which mediate bronchodilatation were shown to differ pharmacologically from those which mediate cardiac actions, and hence they were classified as β_2- and β_1-receptors, respectively (234, 235). Subsequently, α-adrenoceptors were also subdivided since they were shown to be present not only on smooth muscle cells ("postjunctional" receptors) but also on nerve terminals ("prejunctional" receptors) which innervate the smooth muscle. From the effects of various agonists and antagonists, it was found that post- and prejunctional receptors have different properties, and thus, they were subdivided into α_1- and α_2-receptors, respectively (236).

It became clear, however, that both α_1- and α_2-receptors, as well as β_1- and β_2-receptors, pharmacologically defined, are present in the postjunctional smooth muscle membrane (414). Therefore, the different types of receptors cannot be subdivided by their location, but they are now classified according to their relative responsiveness

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to agonists and to antagonists. For example, α_1-receptors are more powerfully activated by phenylephrine than by clonidine, and the reverse is true for α_2-receptors. The responses mediated by α_1-receptors are antagonized by prazosin or phenoxybenzamine, while those mediated by α_2-receptors are antagonized by yohimbine or rauwolscine. Similarly, examples of agonists and antagonists for β_1-receptors are trazolol and practolol, respectively, while those for β_2-receptors are fenoterol and ICI 118, 551 (erythro-*dl*-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol), respectively (see Table 1).

This differentiation of the adrenoceptors may have a functional significance in that the α_1- and β_1-receptors are likely to be located postjunctionally near the site where the neurotransmitter (norepinephrine) is released from the sympathetic nerve terminals and that the α_2- and β_2-receptors are located extrajunctionally (and prejunctionally) being particularly sensitive to the hormone (epinephrine) released from the adrenal medulla (16, 237, 238). However, this hypothesis is likely to oversimplify the actual situation because there are many exceptions.

The identification of the individual receptors has been based, in the first place, on the mechanical response evoked by a given agonist and on the abolition of this response by a specific antagonist. However, the specificity of these agents is often not sufficiently high for a clear differentiation of receptor types. Another complication arises from the different sites of action in smooth muscle preparations which contain other tissue components such as nerve fibers, ganglion cells, and endothelium. An indirect action through these components may influence the direct action of catecholamines on the particular smooth muscle cells under investigation. It is known, for example, that, after blocking α_1- and β-adrenoceptors, norepinephrine exerts a relaxing effect in some vascular muscles mediated through α_2-adrenoceptors located on the endothelium (15a). In the intestine and the myometrium, the activity of one muscle layer may be influenced by the activity of an adjoining muscle layer. If the effectiveness of catecholamines on the longitudinal layer differs quantitatively and/or qualitatively from that on the circular layer, a complex result can be obtained which may lead to confusion.

The distribution of receptors in smooth muscles may depend on the density of innervation and on the distance between the adrenergic nerve terminals and the muscle cell membrane. In several blood vessels from the rabbit, it has been shown that the sensitivity to externally applied norepinephrine is higher in densely innervated vessels than in sparsely innervated ones, even after blockade of neuronal uptake and of β-receptors (33a, 251a). In vascular muscles (*dog* mesenteric artery and saphenous vein), α-receptors seem to be located more closely to the nerve endings compared with β-receptors, because inhibition of neuronal uptake by cocaine potentiated α-effects of catecholamines more than β-effects (152a). This conclusion is supported by the observation that the concentration of phentolamine and prazosin required to inhibit nerve-mediated contractions was about 5 to 7 times higher than that required to inhibit the contraction produced by applied epinephrine or nor-

TABLE 1
Glossary of compound designations

Compound designation	Generic or chemical name
A23187	Calimycin
AA 497	5-Hydroxymethyl-6-hydroxy-2-isopropylamino-1,2,3,4-tetrahydronaphthalene-1-ol
BE 2254	2-(β-(4-Hydroxyphenyl)ethylaminomethyl)tetralone
BHT 920	6-Allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo(4,5-*d*)azepin dihydrochloride
D 600	Gallopamil
FPL 55712	7-[3-(4-Acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4*H*-1-benzopyran-2-carboxylate
$H_{35/25}$	*dl*-Erythro-4-methyl-α-(1-isopropylaminoethyl)benzyl alcohol hydrochloride
ICI 118,551	Erythro-*dl*-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol
M-7	2-N,N-Dimethylamino-5,6-dihydroxyl-1,2,3,4-tetrahydronaphthalene
Quin-2	2-Methyl-6-methoxy-8-nitroquinoline
Ro 20-1724	4-(3-Butoxy-4-methoxybenzyl]-2-imidazolidone
Sgd 101/75	Indanidine
SK & F 525A	Proadifen
SK & F 87696	5,8-Dimethoxy-2-aminotetralin
SK & F 88254	8-Methoxy-2-aminotetralin
SK & F 88444	5,8-Dimethoxy-N,N-dimethyl-2-aminotetralin
SK & F 89748	*l*-1,2,3,4-Tetrahydro-8-methoxy-5-(methylthio)-2-naphthalenamine
SQ 20,009	1-Ethyl-4-(isopropylidenehydrazino)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid, ethyl ester
St 587	2-(2-Chloro-5-trifluoromethylphenylimino)imidazoline
TEA	Tetraethylammonium
UK 14304	2-(8-Bromoquinoxalyl-7-imino)-imidazoline tartrate
WB 4101	2-[(2′,6′-Dimethoxy)-phenoxyethylamino]methylbenzodioxane

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epinephrine, whereas propranolol was equipotent in reducing β-effects of nerve stimulation and of applied epinephrine (152b).

Most smooth muscles seem to contain several subtypes of adrenergic receptors, but at the moment it is difficult to define whether different types are present on the same muscle cell or whether they occur on different cells within small regions, and whether receptors are distributed homogeneously or whether they occur in clusters. Furthermore, it is still not certain that the α_1- α_2-, β_1-, and β_2-receptors are the final classification for adrenoceptors. For example, there is some evidence for adrenoceptors which are apparently resistant to adrenoceptor blocking agents, as described in section VII (168, 169, 282). Since receptors have probably more than one binding site and are linked to intracellular mechanisms regulating cell function, it is quite possible that, due to the presence or absence of certain metabolic pathways, specific enzyme systems, second messengers, etc., slightly different mechanisms operate to produce the mechanical response.

III. Catecholamine Action

Contraction of smooth muscle is thought to be the result of actin-myosin interaction caused by phosphorylation of myosin light chain. The light chain is phosphorylated when myosin light chain kinase is activated by calmodulin and Ca. Thus, an increase of the intracellular free Ca concentration leads to contraction. When the intracellular Ca concentration is decreased, the muscle relaxes due to dephosphorylation of the light chain mediated by phosphatase. The final effect of catecholamines, contraction or relaxation, is thus mainly produced by modulation of these processes, chiefly by a change of the intracellular free Ca concentration. The diversity of the properties and also of the mode of action of catecholamines in different smooth muscles is mainly determined by the way in which the intracellular Ca concentration is regulated in each individual tissue.

For the excitatory action (contraction), catecholamines may increase Ca influx, release Ca from intracellular stores, and/or suppress Ca extrusion or sequestration. When the muscle is electrically excitable, Ca influx occurs during the action potential, whose configuration is modulated directly through mechanisms acting on ionic channels or indirectly through changes in membrane potential. In less excitable, or poorly excitable, muscles, activation of adrenoreceptors may increase Ca influx through the plasma membrane, without generating action potentials, and/or it may release intracellular Ca, which is probably mediated by intracellular second messengers, as discussed in section IX.

The relaxation by catecholamines can also be caused by several different mechanisms, such as suppression of Ca influx, Ca extrusion from the cell, sequestration of Ca into intracellular stores, and/or interference with the contractile machinery. Ca influx can not only be directly blocked by closing Ca channels, but it can also be suppressed indirectly by blocking the generation of action potentials, for example, as a result of hyperpolarization of the membrane due to an increase in K conductance. The contribution of each of these processes seems to vary greatly from one muscle type to another and also depends on experimental conditions. Although various hypotheses have been presented for the action of catecholamines, the actual analysis is still rather superficial, mainly due to limitations of available techniques.

For electrophysiological observations, the sucrose-gap method has often been used. This is a very useful method when muscle fibers are electrically well coupled and their properties are homogeneous. One must be aware, however, that the electrical changes are average values of many cells. Therefore, if the receptor distribution in the recording region is uneven, the conclusion derived from results recorded with the sucrose-gap method may not be reliable and, to obtain more precise information, it is necessary to record intracellularly. It is often assumed that the membrane effect of catecholamines can be neglected in depolarized preparations in excess K medium. However, the important factor is not the change in membrane potential, but the change in Ca conductance of the membrane. The Ca channels, which are not inactivated by depolarization (410), may still be affected by catecholamines even in the depolarized condition without significant alteration of the membrane potential. Thus, one must realize the limitations of each method and be cautious in interpreting the results.

Most of the information available in the literature is concerned with electromechanical coupling. Hence, the main part of this review (sections IV to VII) describes the coupling of receptor activation and contractility through modulation of membrane activity and of the movement of Ca across the cell membrane. The pathways of the intracellular translocation of Ca by second messengers, i.e., the biochemical coupling mechanisms, are described separately in the two final sections VIII and IX.

IV. Spontaneously Active, Highly Excitable Muscles

A. The Taenia of Guinea Pig Caecum

1. α-Action. The longitudinal muscle of the gastrointestinal tract is highly excitable and spontaneously active. This activity is suppressed not only by activation of β-receptors but also of α-receptors. A typical example is seen in the taenia of *guinea pig* caecum (often called the taenia coli), which has been most extensively studied. In this muscle, activation of the α-receptors by epinephrine causes hyperpolarization of the membrane by increasing K (and probably Cl) conductance and cessation of spontaneous activity resulting in relaxation (55, 199, 200, 374). This action is mediated by α_1-receptors, because it is mimicked by phenylephrine and methoxamine, but not by clonidine, and it is abolished by α_1-selective

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blockers (carbidine or phenoxybenzamine), not by an α_2-selective blocker, yohimbine (27, 420).

The initial phase of hyperpolarization of the membrane caused by epinephrine is not much affected by removal of the external Ca, but the late phase disappears within 2 to 3 min during continued application of epinephrine (104, 105, 415, 417). After prolonged exposure (20 min) to Ca-free solution, the early phase is also abolished, and epinephrine has no effect. Mn and La also suppressed more effectively the late phase than the early phase. Following treatment with Ca-free, excess (6 to 12 mM) Mg solution, readmission of Ca increased the membrane resistance in the absence of epinephrine, but it decreased the membrane resistance in the presence of epinephrine (59). These results suggest that epinephrine facilitates Ca influx through the plasma membrane, resulting in increased K and Cl conductances, and that the early transient component of the hyperpolarization observed in Ca-free solution may be due to Ca released from some binding sites (58, 103, 415, 417).

Epinephrine increased Ca loss, and this loss disappeared in Ca-free solution (105). The K efflux, represented by ^{86}Rb efflux, was also increased by epinephrine, and this was blocked by the bee toxin, apamin. When Ca was replaced with Ba, the effect of epinephrine in suppressing the mechanical activity (450) and in reducing the membrane resistance and suppressing the spike activity disappeared (57). The action of Ba was partly antagonized by increasing external Ca. Apamin (103, 257, 258) and TEA (tetraethylammonium) (29) abolished the hyperpolarization induced by α-receptor activation or converted the response to depolarization, probably by blocking the increase in K conductance. The relaxation caused by amidephrine (α-agonist) was also diminished by 1 to 3 nM apamin (20). When the preparations were exposed to K-free solution for 4 to 5 hr, the membrane was depolarized to a range between −10 and 0 mV. Under these conditions, instead of producing hyperpolarization and relaxation, norepinephrine (1 μM) caused a transient depolarization accompanied by contraction still in K-free medium, suggesting an increase in Ca influx (18).

The mechanism by which the K (and possibly Cl) conductance is increased by activation of α-receptors is still a matter of speculation, but the above results all seem to support the hypothesis that Ca may increase the K conductance from the inside of the plasma membrane, as is known for the Ca-activated K conductance in many other cells, and that activation of α-receptors mobilizes Ca into this active site from some binding sites at the outer surface of the membrane, from the external medium, and possibly also from some intracellular store (58, 103, 414, 416). In recent experiments with the patch-clamp method, supporting evidence for the contribution by Ca-activated K conductance to the α-inhibitory action has been obtained (H. Tokuno and T. Tomita , unpublished observations). When norepinephrine (1 μM) was applied to the outside of a single cell, the probability of open state of the Ca-activated K channels recorded under the cell-attached condition was greatly increased with little change in the single channel conductance. This effect was the same as that of increasing the intracellular Ca concentration.

2. β-Action. Activation of β-receptors by isoproterenol causes relaxation by suppressing the spike activity accompanied by a small hyperpolarization (56). This action is abolished by propranolol (a nonselective blocker), but only partially by practolol (β_1-blocker). A β_1-agonist, tazolol, is inactive, suggesting the main involvement of β_2-receptors in the suppression of spike generation (27). A similar conclusion has been reached by using a β_2-selective agonist, AA 497 (5-hydroxymethyl-6-hydroxy-2-isopropylamino-1,2,3,4-tetrahydronaphthalene-1-ol) (216). The suppression of spike activity by AA 497 is markedly inhibited by butoxamine (β_2-selective antagonist), but not by practolol. In addition to this mechanism at the membrane mediated by β_2-receptors, isoproterenol seems to activate also β_1-receptors, which influence intracellular mechanisms causing relaxation (216). The involvement of β_1-receptors can easily be demonstrated in the depolarized condition in excess K medium. Stimulation of adenylcyclase, which is coupled to the β-receptor, results in an increase of the intracellular cyclic AMP level, as described in section VIII.

In the *guinea pig* taenia, both the α- and the β-action are inhibitory. The electrophysiological analysis of the underlying mechanisms, using the sucrose-gap method, has shown that both suppressed the spontaneous spike discharge. However, the hyperpolarization and the decrease of membrane resistance caused by β-receptor activation were either absent or much smaller than those caused by the α-action (52, 56).

Recently, it was found with intracellular microelectrodes that isoproterenol, through activation of β-receptors, causes a substantial hyperpolarization (about 70% of that caused by epinephrine at the same concentration of 0.3 μM through activation of α-receptors). Only occasionally, when a cell was not spontaneously active, isoproterenol failed to cause a change in membrane potential, while epinephrine in the same cell invariably produced a large hyperpolarization. The hyperpolarization caused by isoproterenol is likely to be due to an increase in K conductance, like that caused by α-receptor activation, because it is significantly increased by removing the external K (416). Supporting evidence for an increase in K conductance is the observation that, during the hyperpolarization induced by isoproterenol or epinephrine in K-free solution, K-readmission depolarized the membrane, and that TEA (10 mM) or Ba (2.5 mM), which are both known to decrease K conductance, abolished the catecholamine-induced hyperpolarization. In the absence of catecholamine, the readmission of K, after exposure to zero K, hyperpolarizes the membrane due to

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activation of the electrogenic Na pump (68, 418). The discrepancy between the earlier results obtained with the sucrose-gap method and the recent observations with intracellular recording might be due to the possibility that β-receptors are not as homogeneously distributed as α-receptors, but that they are confined to scattered spots where spontaneous activity is generated. This uneven distribution of the receptors may attenuate the hyperpolarization and the conductance change when measured with the sucrose-gap method.

Since isoproterenol increased ^{45}Ca efflux by about 20% without affecting ^{45}Ca influx, it has been suggested that the β-action is due to activation of an electrogenic Ca pump at the plasma membrane, which accounts for a small hyperpolarization observed with the sucrose-gap method (52). An involvement of cyclic AMP in the β-action (see section VIII) is suggested by the observation that the total tissue Ca did not change during relaxation caused by a phosphodiesterase inhibitor (Ro 20-1724, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidone), which mimicked the isoproterenol relaxation, indicating that the β-action is partly due to an increase in Ca sequestration (302). A contribution by Ca sequestration has also been suggested by a study on the carbachol-induced contractions in Ca-free solution (70). The *guinea pig* taenia produced a transient contraction when carbachol (1 μM) was given after 3-min exposure to Ca-free solution (5.9 mM K, 2 mM EGTA), following treatment with 42 mM K solution containing 1.5 mM Ca to fill intracellular Ca stores. This contraction was increased by about 40% when isoproterenol (1 μM) was added during the Ca-filling procedure in excess K solution. This result was interpreted to indicate that the Ca uptake by intracellular stores was facilitated by isoproterenol, and thus a larger amount of Ca became available for the subsequent stimulation by carbachol in Ca-free solution. The final agreement on which of the mechanisms involved in the intracellular Ca regulation plays the main role in the β-effect should be reached by further experiments.

The relaxing effect of isoproterenol is reduced by depolarization with excess K, but the degree of reduction depends on several factors, such as the Na concentration remaining, the duration of exposure, and the concentration of isoproterenol. There are apparent discrepancies among the publications on the effect of isoproterenol in depolarized preparations, but the reason is often not clear because the experimental conditions are usually not fully described. In the *guinea pig* taenia, during exposure to excess (24 mM) K solution, when observed with the sucrose-gap method, 0.4 μM isoproterenol produced nearly complete relaxation without reducing spike frequency or amplitude (216), confirming similar previous observations using 35 mM K (444). On the other hand, verapamil decreased the spike frequency and caused relaxation roughly in parallel (217). It was therefore concluded that the relaxation caused by isoproterenol was not simply due to a direct effect on spike activity (Ca influx), but on some intracellular mechanism. However, when intracellular microelectrodes were used, such a dissociation between spike activity and relaxation was not clear (H. Tokuno and H. Tomita, unpublished observations).

The relaxation of the taenia through β-receptor activation is reduced by lowering the external Na concentration, but its degree varies to some extent in different experiments. When spike activity was blocked by increasing the external K, norepinephrine no longer caused relaxation, but isoproterenol still reduced the tension. However, the presence of some (about 15 mM) Na and Cl was necessary for the relaxant action of isoproterenol and, furthermore, Ca could not be substituted by Sr or Ba for this action (262). In general, isoproterenol (0.4 to 100 μM) failed to relax the taenia when NaCl was completely replaced by KCl (leaving 6 mM Na as $NaHCO_3$) (135, 183), although in some experiments using a similar solution, a clear relaxation (but smaller than the control) was also demonstrated, for example, in the taenia and the circular muscle of *rabbit* colon (13, 14).

The disappearance of the isoproterenol effect in Na-free solution was confirmed and considered to be partly due to suppression of the Na pump, because ouabain also reduced the relaxant effect (442). However, in K-free medium, isoproterenol was still very effective in causing relaxation (442). During the early stage of ouabain (50 μM) treatment, the effect of isoproterenol was even potentiated, although after about 30 min it was finally abolished, probably not due to blockade of the Na pump, but to an indirect effect, for example, excessive accumulation of Na and/or loss of intracellular K (52). No contribution by the Na pump or by Na-Ca exchange to the β-adrenergic relaxation was also suggested by observations that isoproterenol could still produce relaxation in the presence of ouabain and in Na-free, K-substituted medium (302). However, in this experiment Na was only removed for 3 min before isoproterenol was applied. If Na removal had been prolonged, the relaxant effect of isoproterenol might have been markedly reduced.

There is a report of a clear inhibitory effect of isoproterenol in Na-free solution (323). In this experiment, the preparation was first exposed to Ca-free, excess K Tyrode (Na-free) solution and, at intervals of 10 min, 2.0 mM Ca was readmitted for a short time, 2 min, to produce a contraction. When Ca was readmitted in the presence of isoproterenol (0.5 μM), the Ca-induced contraction was about 30% smaller. It is likely that the relaxing effect of isoproterenol is reduced when the intracellular Ca accumulation is excessive. Thus, if isoproterenol had been applied after a more prolonged application of Ca in Na-free excess K solution, the effect of isoproterenol might have been negligible. It was found that, when isoproterenol had become ineffective in Na-free medium, its relaxing effect was restored by reducing

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the external Ca concentration (K. Baba, S. Nakayama, and T. Tomita, unpublished observation). Complete removal of external Na or prolonged treatment with ouabain may interfere with the intracellular Ca regulation through β-receptor activation due to excessive intracellular Ca accumulation.

B. Small and Large Intestine

1. α-Action. The longitudinal muscle layer of the intestine has similar properties as the taenia (37, 38, 406). It produces spike-type action potentials, and the spontaneous activity occurs in bursts. When the muscle is isolated and the underlying circular layer is removed, spontaneous activity is reduced and may stop. Activation of α-receptors in the longitudinal muscle of small intestine produces an inhibitory effect. In the *guinea pig*, the main action of catecholamines through α-receptors is likely to be the suppression of acetylcholine release from cholinergic nerve fibers by activating the α_2-subtype (48, 221, 333, 447). However, there is also a direct inhibitory action on the smooth muscle cells (45b, 48, 445). In the proximal and terminal ileum of *guinea pig*, the relaxant action of norepinephrine was antagonized by prazosin and phenoxybenzamine in concentrations that have no effect on acetylcholine release, suggesting the presence of inhibitory α_1-receptors (125, 126). On the other hand, Bauer (26) obtained some evidence that the inhibitory postjunctional effect of epinephrine or ephedrine on the proximal ileum was mediated through α_2-receptors, mainly because it was inhibited by yohimbine. The presence of inhibitory α-receptors in the smooth muscle cells has also been demonstrated in the *rabbit* jejunum using the denervated longitudinal muscle. This muscle, contracted by acetylcholine, was relaxed by phenylephrine, norepinephrine, or oxymetazoline in the presence of a β-blocker, sotalol, and the relaxation was blocked by dibenamine (447).

The mechanism of action of catecholamines in the small intestine has not been as much investigated as in the *guinea pig* taenia. Using intracellular microelectrodes, it was demonstrated, in the longitudinal muscle of *guinea pig* proximal ileum, that in the presence of propranolol, norepinephrine (1 μM) hyperpolarized the membrane by about 10 mV, decreased membrane resistance, and stopped the spike activity (28). This effect was not affected by prazosin (1 μM), but converted to a weak depolarization with acceleration of spike activity by yohimbine, supporting the observation that α_2-receptors are responsible for the inhibitory action (26). Thus, the inhibitory action of catecholamines in the small intestine, mediated by α-receptors, is apparently similar to that observed in the taenia but, in the small intestine, it seems to be mediated by a different subtype, the α_2-receptor in contrast to the α_1-receptor in the taenia.

The electrical activity of the longitudinal muscle of *rabbit* jejunum consists of slow waves and spikes superimposed on the slow wave, and the mechanical activity is correlated with the spike component. Epinephrine (1 μM) abolished the spike and contraction without affecting the slow wave. This effect was followed by hyperpolarization and slight increase in slow wave amplitude. The hyperpolarization was still observed in the absence of K ions but blocked by Cl removal (propionate substitution) (123). It was considered that the hyperpolarization is due to an increase in K conductance and that this depends on the presence of Cl.

In the longitudinal muscle layer of the *rabbit* colon, replacement of Ca with Ba (4 mM) produced contraction. This contraction was not suppressed by phenylephrine (5 μM) (10). Similarly, the maintained contraction of the longitudinal muscle of *rabbit* jejunum, caused by 5 mM Ba in the absence of Ca, was not affected by phenylephrine (10 μM), but in the presence of 2.5 mM Ca, the contraction by 1 mM Ba was relaxed by phenylephrine (10 μM) through activation of α_1-receptors. When the Ba concentration was increased, relaxation by phenylephrine became transient and was followed by contraction. At concentrations of 10 to 15 mM Ba, phenylephrine produced only contraction. In 5 mM Ba and a low Ca concentration (0.2 to 2 mM), only contraction was elicited by phenylephrine, but when Ca was increased to 5 mM, this response was changed to the normal relaxation. A high concentration of Mg (20 mM) preferentially blocked the contractile response. Essentially the same results were obtained with the *guinea pig* taenia. Thus, it was concluded that Ca is essential for both the relaxant and the contractile response mediated through α_1-receptors, but that Ba and Mg modify the response (450). It is not certain from these experiments, however, whether Ba blocked the inhibitory response uncovering the remaining excitatory response or whether Ba converted the inhibitory effect to an excitatory effect by blocking the increase in K conductance.

In the small intestine, in addition to the inhibitory effect, an excitatory effect of α-receptor agonists can also be demonstrated, particularly in the terminal ileum. The contraction induced by norepinephrine, epinephrine, or phenylephrine in the presence of atropine and a β-blocker, sotalol, was antagonized by phenoxybenzamine and dibenamine, but not by yohimbine (25, 448, 449). Clonidine and oxymetazoline produced only weak contractions. These results indicate that α_1-receptors are responsible. The excitatory response varies with the distance from the ileocaecal junction. Norepinephrine and epinephrine produce contraction mediated by the α_1-postjunctional receptors up to 20 cm from the junction, although the contraction by phenylephrine can be demonstrated throughout the ileum with stronger potency towards the junction (25, 26). Using intracellular recording, it was shown that, in the longitudinal muscle of the terminal part of *guinea pig* ileum, norepinephrine and phenylephrine depolarized the membrane and increased

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the spike frequency, accompanied by a decrease in membrane resistance, through activation of α_1-receptors (28).

2. β-Action. The activity of the longitudinal muscle of *guinea pig* ileum is suppressed through activation of the β-receptors located on the smooth muscle cells (48, 147, 221). When various agonists and antagonists were tested in the middle region of the ileum, contracted by 100 mM KCl, it was found that relaxation was mediated only through the β_1-receptor subtype (147). The same results were obtained in the *rabbit* upper intestine (duodenum and jejunum) (234, 235). However, other experiments revealed the existence of the β_2-receptor subtype in addition to β_1-receptors. In the *rabbit* ileum, only β_1-receptors seem to be activated by epinephrine and norepinephrine, because the inhibitory action was blocked by metoprolol, a β_1-selective blocker, not by $H_{35/25}$ (*dl*-erythro-4-methyl-α-(1-isopropylaminoethyl)benzyl alcohol hydrochloride), a β_2-selective blocker. On the other hand, isoproterenol is able to stimulate both β_1- and β_2-receptors (438). According to the effects of methoxamine, isoproterenol, salbutamol (β_2-agonist), and dobutamine (β_1-agonist), the β-receptors of the large intestine of the *rabbit* are predominantly β_2-type, and those of the small intestine are predominantly β_1-type (377).

In the longitudinal muscle of *guinea pig* ileum, studied with intracellular microelectrodes, isoproterenol (0.3 to 1 μM), by activating β-receptors, decreased the frequency of spontaneous spikes without changing the membrane potential and membrane resistance (28). It would be interesting to investigate whether in the ileum there are some spots where activation of β-receptors produces a significant hyperpolarization as in the taenia, but whether they are less densely distributed than in the taenia.

The postjunctional β-receptor in the *rat* and *cat* colon was found to be the β_2-subtype, and the inhibitory mechanism involved in the enteric nerves was mediated through the β_1-subtype (122).

The mechanism underlying the action of catecholamines on intestinal smooth muscles have not been analysed to the same extent as in the taenia of the caecum. The fundamental mechanisms causing relaxation by β-receptor activation are probably the same in all gastrointestinal muscles. But the importance of changes in membrane function in relation to modification of intracellular regulatory processes may be quite different in different regions of the gastrointestinal tract and may also be different in the longitudinal compared with the circular muscle layer.

C. Myometrium

The activity of the myometrium is influenced by hormonal conditions, or gestation, and varies in different species. Furthermore, the pattern of activity in the longitudinal layer differs from that in the circular muscle layer; e.g., the configuration of the action potential is a spike type in the former but a plateau type in the latter. When one investigates the action of catecholamines, these factors must be taken into consideration. Catecholamines inhibit the myometrium only through β-receptors, while the activation of α-receptors causes contraction. The proportion in which the two receptor types occur varies in different species and also in different hormonal conditions (270, 271), and recently it became clear that catecholamine effects in longitudinal muscle are significantly different from those in the circular muscle layer.

1. α-Action. Binding of [^{3}H]dihydroergocryptine (α-antagonist) to α-receptors was 3 times greater in the myometrium from estrogen-treated *rabbits* than from *rabbits* treated with estrogen followed by progesterone, suggesting that the number of α-receptors increases with estrogen domination (346, 347, 452). This change was found to be due to an increase in α_2-receptors (173). There was no change in the number of α_1-receptors, and the contractile response was coupled only with α_1-receptors, not with α_2-receptors, explaining the finding that estrogen treatment did not change the sensitivity of the myometrium to norepinephrine or phenylephrine. This observation is in accord with the result obtained in the circular muscle of the *guinea pig* myometrium (2). The significance of the increase in α_2-receptors obtained by the radioligand study remains to be clarified in relation to the two different muscle layers.

In the preparturient (the 22nd day) *rat* myometrium, the relative population of α_1- and α_2-subtypes was assessed by studying competition between [^{3}H]dihydroergocryptine and selective agonists and antagonists, or by using [^{3}H]prazosin and [^{3}H]rauwolscine (267). The results indicated that the membrane fraction obtained from a mixture of longitudinal and circular muscles contained 45% α_1-receptors and 55% α_2-receptors. However, their modification by pregnancy has not been analysed.

In the circular muscle of the pregnant *rat* myometrium, α-receptors predominate, and norepinephrine increases spontaneous contractions. The sensitivity of circular muscle to the stimulant action of norepinephrine declined during mid-pregnancy, and at term, norepinephrine (1 μM) inhibited contractions, probably due to an increase in sensitivity and/or the number of β-receptors (82). In the mid-pregnant (12- to 15-day) *rat* myometrium, the α-excitatory action was clearly observed only in the circular muscle whose action potential consists of spike and plateau phase. Phenylephrine (0.5 μM) prolonged the duration of the plateau potential leading to an increase in the mechanical response, but the membrane potential, membrane resistance, and the amplitude of plateau were apparently not affected (213, 214). With norepinephrine, a slight depolarization of the membrane and increase in frequency, duration, and amplitude of the plateau phase of action potentials were observed (9 to 20 days) (219). Some depolarization accompanied by a decrease in membrane resistance was also observed

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during excitation caused by norepinephrine or phenylephrine in the 21-day-pregnant *rat*, suggesting an increase in Ca and/or Na conductance (331). Although an inhibitory β-action was predominant in the longitudinal muscle, the excitatory α-action appeared with a low concentration (0.1 μM) of norepinephrine during and 1 day after the delivery (219).

Since the plateau potential was very sensitive to the external Ca concentration and the effect of phenylephrine could be demonstrated in Cl-deficient solution, α-receptor stimulation was considered mainly to increase the Ca conductance, causing prolongation of the plateau and increasing contraction (213, 214). When Na was replaced by choline, leaving 15.7 mM Na as $NaHCO_3$ buffer, the membrane was initially depolarized to the plateau level, but slowly the action potential reappeared with a prolonged plateau. At this stage, the effect of epinephrine was nearly abolished, suggesting an involvement of Na conductance in the control of Ca channels opened by α-receptors.

In the longitudinal muscle of estrogen-dominated *guinea pig* myometrium, α-receptor stimulation produced depolarization with a decrease in membrane resistance and increased spike frequency. The depolarization and the decrease in membrane resistance could still be observed in the presence of La (1 mM). The depolarization was probably mainly due to an increase in Cl conductance, because it was abolished by replacing Cl with glutamate, benzene-sulphonate, or isethionate, but not by replacing Na with Tris. However, Na was necessary for the increased spike activity (54, 408).

Since the spike configuration is different between longitudinal (spike-type) and circular muscle (plateau-type), it is possible that, in the longitudinal muscle, activation of α-receptors is mainly mediated by slow depolarization of the membrane (due to an increase in Cl conductance) which leads to an increase in spike frequency, while in the circular muscle, it is mediated by prolongation of the plateau of action potentials (due to an increase in Ca conductance).

2. β-Action. In most species, the β-receptors in the myometrium are mainly of β_2-type (*rat*: 44, 234, 235; *guinea pig*: 229, 319). In the progesterone-primed *rat*, β_2-receptors predominate, but the presence of β_1-receptors can also be demonstrated in estrogen-primed *rat* (204). In the *rat*, the β-inhibitory effect increased in the circular muscle at term (78, 82, 219). In the longitudinal muscle, either no significant change (82) or an increase in the sensitivity to β-agonists (78) was found at the end of pregnancy. In the longitudinal muscle of the *guinea pig*, the relaxing effect of epinephrine through β-receptors was significantly greater after treatment with estradiol and progesterone (2). In the *rabbit*, β-receptors decreased markedly at the end of gestation, and this agreed with the binding studies using [^{3}H]dihydroalprenolol (β-blocker), although no discrimination was made between longitudinal and circular muscle layers (424). However, in other studies with [^{125}I]iodohydroxybenzylpindolol (β-blocker), the number of β-adrenergic binding sites in the *rabbit* myometrium was not changed by treatment with estrogen or with estrogen followed by progesterone (346, 347). This suggests that the hormonal influence is on some process beyond the agonist-receptor interaction.

In the longitudinal muscle of pregnant *rat* myometrium, studied by microelectrodes, the relaxation caused by isoproterenol (0.4 μM) was accompanied by a considerable hyperpolarization (about 12 mV) of the membrane. Isoproterenol decreased the tissue Ca content in the myometrium contracted by excess K, measured with the lanthanum method (273). However, an increase in ^{45}Ca efflux could not be demonstrated because of the high background of Ca exchange, probably due to the enormous contribution of extracellular binding sites to the Ca exchange (228). The degree of hyperpolarization was not affected by removal of K or Cl, but reduced by about 50% by ouabain (1 mM). Reducing the temperature to 10°C also decreased the hyperpolarization and increased the latency of the onset of hyperpolarization. From these results, activation of an electrogenic Ca pump was suggested for the hyperpolarization caused by isoproterenol, in addition to a contribution by the Na pump (273). In subsequent studies, however, it was found that the hyperpolarization by isoproterenol was inversely proportional to the external K concentration and accompanied by a decrease in membrane resistance. Thus, an increase in K conductance was proposed as the underlying mechanism for the hyperpolarization (226). The hyperpolarization accompanied by a reduction of membrane resistance during activation of β-receptors was confirmed in the longitudinal muscle of pregnant (13- to 19-day) *rat* myometrium (214).

The circular muscle of estrogen-treated *rat* myometrium was also hyperpolarized with a reduction of membrane resistance in response to isoproterenol (10 nM) (330). At a lower concentration (1 nM), isoproterenol suppressed the plateau of the action potential without much change in membrane potential. These effects of isoproterenol were observed in Locke solution containing no Mg, but they were slowly potentiated when 0.5 mM Mg was added. Similarly, in the circular muscle of pregnant (13- to 19-day) *rat* myometrium, isoproterenol (0.4 μM) suppressed the action potential with a decrease in membrane resistance when studied with the sucrose-gap method (214, 329). Thus, the β-action on the plasma membrane may be fundamentally the same in the myometrium and the taenia in increasing the K conductance of the plasma membrane, and the only difference may be the density or homogeneity of β-receptor distribution.

Recently, the effects of cycloheximide (a protein synthesis inhibitor) on the circular muscle of pregnant *rat* myometrium have been reported (276). In this muscle, norepinephrine (0.1 to 0.3 μM) had an excitatory effect

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on the 18th day of pregnancy, but this was converted to inhibition on the 22nd day. This alteration was prevented when the *rat* was treated with cycloheximide (50 μg) daily from the 18th day. In the preparation freshly isolated on the 21st day, norepinephrine evoked an excitatory response, which became inhibitory after prolonged exposure to Krebs solution for more than 7 hr. This in vitro conversion of the response was also prevented by application of 6 μM cycloheximide to the incubation solution. The results suggest that protein synthesis is involved in the appearance of β-receptor dominance in the late stage of pregnancy.

In the pregnant *mouse* myometrium, isoproterenol (1 μM) also hyperpolarized the membrane (by about 15 mV) and blocked spontaneously generated spikes, but a spike of normal amplitude could be evoked by electrical stimulation. No obvious change of membrane resistance was detected during the hyperpolarization, but due to technical difficulties in measuring the membrane resistance, this may not be conclusive. The inhibitory effect of isoproterenol could still be demonstrated even after 1 to 2 hr of exposure to Na-free (Tris-substituted) solution. It seems that the relaxation mediated by β-receptors in the myometrium is more resistant to Na removal than that in the *guinea pig* taenia. The depolarization and block of spontaneous spike activity caused by Na removal were both restored by isoproterenol, but the presence of Ca was necessary for this action. The results were interpreted to be related to a decrease in the intracellular free Ca concentration caused by isoproterenol (264).

In the pregnant *rat* myometrium, a low concentration (0.01 μM) of isoproterenol stopped the spontaneous spikes without change in membrane potential. The contracture produced by 47.5 mM (80 mM Na) or 127 mM K (0 mM Na) was partially (about 30%) suppressed by 1 μM isoproterenol. This relaxation was not accompanied by hyperpolarization and was not affected by complete removal of the external Na in 47.5 mM K medium. The results indicate that hyperpolarization is not a prerequisite for relaxation mediated through β-receptors (287), supporting previous similar observations (110). It would be interesting to investigate whether, as in the taenia, different subtypes of β-receptors are responsible for the membrane effect and the intracellular effect. Furthermore, it should be clarified whether a causal relation exists between the increase in net Ca efflux and the hyperpolarization with or without a reduction of membrane resistance.

D. Portal Vein

The portal vein is different from most other vascular smooth muscles which are normally electrically quiescent. In the longitudinal muscle of the portal vein, spontaneous spike activity appears in bursts, as in the small intestine (145, 307, 411). This similarity to the longitudinal muscle of the small intestine is probably related to the fact that they have the same embryonic origin (184, 371).

1. α-Action. Pharmacological analysis shows that the portal vein of *rabbit* contains both α_1- and α_2-receptors, although the α_1-receptors predominate (111, 117). In the *canine* portal vein, the main α-receptor was found to be the α_1-subtype (372).

In the *guinea pig* portal vein, phenylephrine, norepinephrine, and epinephrine depolarized the membrane, increased the frequency of burst discharges, and prolonged burst duration (411, 437). According to studies with the sucrose-gap method on the *rat* portal vein, spontaneous activity was increased, and membrane resistance was decreased during depolarization caused by epinephrine or norepinephrine, and the effects of modifying the external ionic composition indicated that this was due to increases in Ca, Cl, and Na conductances (374). Also in the *rat*, norepinephrine (6 μM), by stimulating α-receptors, increased ^{36}Cl efflux by 85% and ^{42}K efflux by 15 to 20%, but had no effect on ^{24}Na efflux (439). Thus, the depolarization was considered to be mainly due to an increase in Cl permeability. Furthermore, the sensitivity to norepinephrine was increased when Cl was replaced with more permeant anions (NO_3 or Br), but the responsiveness was reduced by Cl replacement with less permeant anions (isothionate or benzenesulfonate). These results are also in accord with the hypothesis that norepinephrine increases Cl conductance through α-receptors (440).

In the *rat* portal vein, the contraction caused by 15.5 μM norepinephrine was reduced to 11% by 25 μM verapamil (36). In the *guinea pig* portal vein, the contraction caused by norepinephrine (15 μM) was blocked by removal of external Ca within a few min, and 0.5 to 2 mM La also suppressed the response. Thus, Ca influx was thought to be responsible for the norepinephrine contraction (143). However, when the norepinephrine concentration was high (50 to 500 μM), approximately 40% of the contraction was insensitive to verapamil or D 600 (gallopamil) (5 to 10 μM), a concentration that blocked the K contracture. Verapamil (5 μM) blocked the spike activity but only partially reduced the slow depolarization of the membrane caused by norepinephrine (10 μM) (144). Thus, the spike-independent component of transmembrane Ca influx activated by norepinephrine appeared to be resistant to organic Ca channel blockers. In other experiments, norepinephrine (10 μM) produced contraction in Ca-free medium after treatment with 10 mM caffeine, whereas caffeine failed to evoke contraction after norepinephrine application. Since caffeine is generally assumed to release Ca from the sarcoplasmic reticulum (SR), norepinephrine seemed to release Ca from some other source in addition to the SR (308).

In recent studies with electron probe X-ray microanalysis on the *guinea pig* portal vein, it was found that the amount of Ca contained in the SR was reduced when

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a contraction was evoked by norepinephrine (30 μM) in Ca-free medium containing 3 mM La (42). A contraction of nearly maximum size could be produced repeatedly at 2-min intervals for up to 15 min, provided that norepinephrine was washed out at the peak of contraction. This suggests that Ca released from the SR can recycle intracellularly. It was also shown in the *rabbit* portal vein with the same method that cytoplasmic Ca was significantly increased during maximal contraction caused by norepinephrine or high (80 mM) K (43).

An important role of the intracellular store, probably the SR, has been recognized, not only as a store site which releases and sequestrates Ca, but also as an amplifying apparatus capable of increasing the intracellular Ca concentration. There is some evidence to indicate that Ca entering the cell through the plasma membrane is not directly utilized by the contractile machinery, but acts on the intracellular Ca stores as a trigger of the Ca-induced Ca-release mechanism (233). This is further discussed in section IX.

In the *ferret* portal vein, the intracellular free Ca concentration has been measured with a Ca-sensitive dye, aequorin. Aequorin was loaded into the cells by making the membrane permeable in Ca-free solution, and the membrane was resealed by gradually readmitting Ca. When a sustained contraction was produced by phenylephrine, the aequorin signal increased rapidly but soon declined to a level slightly above the basic level already during the rising phase of the contraction. In contrast, during the K contracture, the light signal was maintained (100, 299). Thus, there is no close correlation between the intracellular free Ca concentration and the tension development caused by phenylephrine. Ca may act only as a trigger of the contractile response, and some other process may increase the effectiveness of Ca in activating the contractile machinery by an as yet unknown mechanism (e.g., through a contribution of protein kinase C (194, 316, 317), as discussed in section IX.

2. β-Action. Not much information is as yet available on the β-action in the portal vein. Isoproterenol, over 0.04 μM, produced hyperpolarization of the membrane and stopped the spike activity in the *guinea pig* portal vein (411). Both the longitudinal and circular muscles of *dog* portal vein, contracted by methoxamine (10 μM) or KCl (50 mM), were rather insensitive to isoproterenol. They were only relaxed by less than 25% with a concentration of 3 μM isoproterenol (215b).

The effect of isoproterenol on the intracellular free Ca concentration was investigated by using aequorin in the *ferret* portal vein contracted by excess K (33 to 46 mM) (300). The results indicated that the cytoplasmic Ca level was not affected or even slightly increased during the relaxation caused by isoproterenol, although a decrease of intracellular free Ca was detected when relaxation was induced by the removal of external Ca or by addition of sodium nitroprusside. Thus, at least in this preparation, isoproterenol is not lowering cytoplasmic Ca levels, but uncoupling the process between Ca ions and force generation, probably at the contractile protein (see section VIII).

The changes of the intracellular free Ca concentration in the *ferret* portal vein, measured by the aequorin method, are fascinating findings for both α- and β-receptor activation, but whether these findings can be applied to the mechanisms involved in adrenoceptor activation in other smooth muscle types is an urgent problem to be solved. It would be also interesting to know the responsible receptor subtype and the relationship between the intracellular free Ca concentration and the electrical activity. Further experiments are necessary with different Ca indicators and other smooth muscles.

E. Summary

In smooth muscles with spontaneous spike activity, catecholamines increase spike frequency to increase the muscle tone through α-receptor activation, and they decrease spike frequency to cause relaxation, either through α- or β-receptor activation. These changes are generally induced by depolarization or hyperpolarization of the membrane, respectively. The ionic mechanism for depolarization is not well established, but a contribution by increased Cl conductance has been considered for the longitudinal muscle of *guinea pig* myometrium and *rat* portal vein. In the circular muscle of myometrium, prolongation of the plateau phase of the action potential also contributes to an increase in tension development, probably mediated through α_1-receptors.

The hyperpolarization mediated through α_1-receptors in the taenia, but probably α_2-receptors in the small intestine, is due to an increase in K conductance. The hyperpolarization caused by β_2-receptor activation in the taenia may also be due to the same mechanism, but further careful studies are necessary. The increase in K conductance in the α-action is due to activation of the Ca-dependent K channel, at least in the taenia. Although phosphoinositides are thought to be involved in activation of α_1-receptors in some smooth muscles (see section IX), this problem has not been investigated in relation to the membrane conductance.

In addition to the membrane phenomena, some intracellular action is also involved in the suppression of contraction, probably mediated through the β_1-subtype. This includes a reduction of the intracellular free Ca concentration by Ca extrusion, Ca sequestration, and/or a direct inhibition of the contractile machinery. Cyclic AMP is considered to be involved in these mechanisms. However, in order to avoid confusion, the contribution of cyclic AMP to the β-action and also the involvement of phosphoinositides in the α-action are described separately in sections VIII and IX.

The correlation between the changes of membrane properties and the changes of intracellular Ca regulation produced by the action of catecholamines is one of the

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most interesting fields to investigate. The relative contribution by these two mechanisms in determining the response to α_1- and α_2- as well as β_1- and β_2-receptor activation should be clarified. It may be that translocation of intracellular Ca to the plasma membrane, caused by the catecholamine, modifies the ionic permeability of the membrane, or that a second messenger acts simultaneously on the intracellular regulatory system as well as on the plasma membrane. But in physiological conditions, the most sensitive response of highly excitable muscles to low concentrations of catecholamines is the modulation of spike activity at the plasma membrane, whereas contractions evoked in experimental conditions by action potential-independent processes are relatively resistant to the inhibitory action of catecholamines.

V. Quiescent, but Highly Excitable Muscles

The vas deferens and the ureter fall into this category. They are normally quiescent, but in vivo the vas deferens is excited by nerve impulses and the ureter by propagated action potentials arising from a pacemaker region in the calix. The vas deferens shows a simple spike-type action potential, whereas the ureter shows a plateau-type action potential.

A. Vas Deferens

The vas deferens is densely innervated, and nerves may release not only norepinephrine but also some other transmitter, such as ATP. The action potential evoked by nerve activity can propagate through electrical coupling between cells, but the range of propagation greatly varies in different species and probably along the vas, due to the different degrees of cell-to-cell coupling.

1. α-Action. In the *rat* vas deferens, the excitatory effects of phenylephrine were abolished by prazosin (6 μM) and WB 4101 (2-[(2′,6′-dimethoxy)phenoxyethylamino]methylbenzodioxane) (0.1 μM) (259), and the pA_2 values for prazosin were 8.00 against norepinephrine and 8.35 against phenylephrine (5). On the other hand, the pA_2 value of yohimbine as an antagonist of phenylephrine was 5.99, which is much smaller than the values (average, 7.82) expected for α_2-receptors (353). These results indicate that α_1-receptors are responsible for the postjunctional excitation by catecholamine. When amidephrine and WB 4101 were used as α_1-agonist and -antagonist, respectively, the pA_2 value in the *rat* vas deferens was very similar (8.82) to that in the *guinea pig* taenia (8.91), suggesting that the same type of α-receptors is probably involved for excitation in the former and for inhibition in the latter preparation (59). The presence of a homogeneous population of α_1-receptors was also demonstrated by studying radioligand binding with ^{125}I-labelled BE 2254 (2-(β-(4-hydroxyphenyl)ethylaminomethyl)tetralone), a highly selective α_1-antagonist (296). Similarly, in the *mouse* vas deferens, yohimbine had no effect on the contraction induced by phenylephrine, although it antagonized the inhibitory effect of norepinephrine and clonidine on the twitch response to electrical stimulation (269).

The vas deferens responds to nerve stimulation with two phases, an initial fast phase and a later slow tonic contraction. In the *guinea pig, rabbit,* and *rat*, the first component was preferentially antagonized by a P_2-purinoceptor antagonist, arylazido aminopropionyl ATP ($ANAPP_3$), or by a stable analogue of ATP (α,β-methylene ATP), and the second component was abolished by prazosin, phenoxybenzamine, or dibenamine (127, 293, 383, 386). In the *rat* vas deferens, the first phasic component of the contraction, resistant to α-blockers, appears predominantly in the prostatic portion, while the second slow component, susceptible to α-blockers, is prominent in the epididymal portion (50, 51). In the *guinea pig* vas deferens, the early phasic response is considered to be mainly due to ATP, whereas in the *mouse*, mainly due to norepinephrine. The late response is evoked mainly by norepinephrine in both species, although ATP may act as a cotransmitter (394).

In the *rat* vas deferens, the contraction evoked by nerve stimulation could not be abolished but was often enhanced by high concentrations of α-blockers, such as phentolamine, piperoxan, thymoxamine, and WB 4101 (59, 119a). This is probably due to the fact that the nerve fibers release ATP and also some other substance (e.g., neuropeptide Y) in addition to norepinephrine. The proportion of these substances may differ in different regions of the vas, in different species, and in different experimental conditions, such as frequency of stimulation (50, 51, 127, 293, 385, 386).

The ineffectiveness of α-blockers on the nerve-mediated response can be theoretically explained by assuming that the "junctional" receptors, which are selectively activated by norepinephrine released from the nerve, differ in their properties from the "extrajunctional" receptors, which have the typical properties of α-receptors and can be easily activated by exogenous norepinephrine (181). However, since evidence for the involvement of ATP as a transmitter is strong, at least in the vas deferens, it may not be necessary to assume two special receptor types for norepinephrine (281), although the concept of "junctional" receptors still holds.

The *guinea pig* vas deferens was depolarized by both norepinephrine and ATP applied into the bath, but the time course was much slower for norepinephrine than ATP. On the other hand, when applied locally for about 20 ms by a micropressure ejection device, ATP produced a rapid depolarization, mimicking the excitatory junction potential (ejp), but norepinephrine failed to produce depolarization (383, 385).

In the *guinea pig* vas deferens, it has been shown with the double sucrose-gap method that electrotonic potentials were reduced during the depolarization induced by norepinephrine (0.5 to 5 μM) and that the depolarization was suppressed by removal of the external Na, but not

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by Cl substitution with benzenesulfonate (261). Thus, it was concluded that norepinephrine probably increases Na and Ca conductances. The decrease of the electrotonic potentials, however, could be a secondary effect due to the large depolarization and the spike activity. According to a recent report in which a similar method was used, the depolarization induced by norepinephrine (50 μM) was associated with an increase in electrotonic potentials, and the norepinephrine-induced depolarization was increased during conditioning depolarization, while the direction of the potential change was reversed when the membrane was hyperpolarized by more than 20 mV. These results suggest that a decrease in K conductance is responsible for the depolarization which leads to the spike generation (441).

In the *rat* vas deferens, the K (160 mM) contracture was much more sensitive to Ca channel blockers [50% inhibitory concentration (IC_{50}) of nifedipine and verapamil, 0.05 to 0.09 μM and 0.6 to 1.5 μM, respectively] than the methoxamine-induced contraction (IC_{50} of nifedipine and verapamil, 2.4 μM and 29.8 μM, respectively) (162, 163). Verapamil (0.03 to 0.1 μM) inhibited noncompetitively the contraction evoked by norepinephrine (1 to 100 μM), but competitively the contraction evoked by Ca in the presence of excess K (96). The prostatic portion of the *rat* vas deferens produced a biphasic response to norepinephrine, which disappeared on removal of the external Ca. The initial part of the response was resistant to Ca channel blockers, but the late part was abolished (164, 395). In the epididymal portion, the entire response was abolished by verapamil (10.2 μM), D 600 (9.6 μM), or nifedipine (1.44 μM). The initial response of the prostatic portion was not affected by nifedipine up to 14.4 μM, but reduced by very high concentrations of verapamil [50% inhibitory dose (ID_{50}), 74.5 μM] and D 600 (ID_{50}, about 100 μM) (164), probably due to unspecific effects. In the *guinea pig* vas deferens, both the action potential and the initial twitch response are abolished by nifedipine (10 to 30 μM), whereas the ejp is nifedipine resistant (36b). The effects of Ca channel blockers should be further clarified in relation to the mechanism of excitation-contraction coupling following receptor activation, in which the turnover of phosphoinositides plays an important role (see section IX).

2. β-Action. In the vas deferens of *rat* (137, 259) and *guinea pig* (137), the presence of inhibitory β-receptors has been demonstrated by the effects of isoproterenol and salbutamol and their abolition by β-blockers such as sotalol, propranolol, and pronethalol. Isoproterenol also had an α-excitatory action. Salbutamol, a β_2-agonist, produced inhibition without any excitatory action (259). The effects of selective agonists (terbutaline, tazolol) and antagonist (atenolol) suggest that the *rat* vas deferens contains only β_2-receptors and no β_1-receptors (254). The presence of a homogeneous population of β_2-receptors has also been demonstrated by using ^{125}I-pindolol binding assays (280).

B. Ureter

In the *guinea pig* ureter, norepinephrine (50 μM) prolonged the plateau of the action potential, without increasing its amplitude, and increased the contraction through activation of α-receptors (373). The resting membrane potential and membrane resistance were either not affected or, occasionally, the membrane was slightly depolarized accompanied by a small increase in membrane resistance. In the absence of Na, the effect of norepinephrine disappeared. Mn (2 mM) blocked the spike component superimposed on the plateau and markedly diminished the contraction, although a slow action potential of plateau type could still be evoked. Norepinephrine produced no effect in the presence of 2 mM Mn. The main effect of norepinephrine was considered to be an increase in the slow Na conductance responsible for the plateau (373). There seems to be some similarity between the α-excitatory action on the ureter and on the circular muscle of myometrium in prolonging the plateau potential, although the underlying ionic mechanism differs, the increase of membrane conductance in the myometrium being mainly for Ca and in the ureter mainly for Na.

C. Summary

In the vas deferens, activation of α_1-receptors by exogenously applied catecholamines, in low doses, can cause contraction with little or no change in membrane potential. High concentrations of norepinephrine cause depolarization and discharge of action potentials. The depolarization is likely to be due to a decrease in K conductance of the membrane. Nerve stimulation releases norepinephrine and ATP, both of which produce contraction through different mechanisms. The ejp is resistant to adrenoceptor blocking agents and is probably caused by ATP; it does not produce a mechanical response. When the ejp reaches threshold, action potentials are triggered which lead to the initial fast component of the contraction. This is resistant to adrenoceptor blocking agents; it is abolished by nifedipine (36b, 164). The role of norepinephrine, simultaneously released from nerve fibers in the nerve-mediated contraction, has not yet been wholly clarified. It may be that norepinephrine has to reach extrajunctional receptors to cause depolarization (like exogenously applied norepinephrine), resulting in the slow component of the contraction. This is abolished by adrenoceptor blocking agents and is abolished by Ca-channel blockers. Depending on the animal species, contraction produced by exogenous norepinephrine may be partly the result of intracellular Ca release, since the initial rapid phase is resistant to Ca-channel blockers (164). However, the contribution of intracellular Ca release to the contraction seems much smaller in the vas deferens, compared with vascular smooth muscle (see

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section VII), because it is quickly abolished by removal of external Ca. The important role of phosphoinositides in the coupling of the α-receptor activation with the contractile response is described in section IX.

In the ureter, the main excitatory effect of α-receptor activation is reflected in the prolongation of the plateau of the action potential, although there is also some indication that α-receptor activation may decrease the resting K conductance. From a functional point of view, it would be interesting to investigate the catecholamine action on the pacemaker activity in the calix region.

The inhibitory β-receptors may exist in quiescent, excitable muscles, but their role is probably less significant compared with that of excitatory α-receptors, and the mechanism of the β-action in these muscles has not much been analysed.

VI. Poorly Excitable Visceral Muscles

In contrast to those smooth muscles that are quiescent but, nevertheless, highly excitable, there are others in which it is very difficult to evoke an action potential. The muscles in the wall of large blood vessels (e.g., the aorta) and in some parts of the stomach, airways, or anococcygeus belong to this category. Many of these muscles have intermediate degrees of electrical excitability and may maintain various degrees of muscle tone, associated with slow fluctuations of the membrane potential. For example, those in the stomach and the trachea of *guinea pig* produce slow potential changes (a few mV to about 40 mV in amplitude), but electrical stimulation generally fails to evoke an all-or-none action potential, although the spike generation is markedly facilitated by TEA, probably by suppressing the K conductance of the membrane.

The properties of vascular smooth muscle differ markedly in different regions (see section VII). The properties of the smooth muscle of the airways have not yet been analysed in detail, but it has been found that the innervation and the receptor density change along the branches of the bronchial tree. Regional differences vary also in different species, and they may be related to the animal behavior (herbivorous, carnivorous, etc.).

A. Stomach

The properties of the stomach muscle are different in the longitudinal and the circular layer and also differ with the region. The fundic part generates an irregular muscle tone, while a slow rhythmic mechanical activity dominates towards the antrum. The muscle tone of the fundic region, at least in the *guinea pig*, is likely to be sustained by endogenous prostaglandins, because it is abolished by indomethacin. The rhythm of activity in corpus and antrum regions is determined by slow regular oscillation of the membrane potential (slow wave), but the strength of contractions is mainly determined by a spike component superimposed on the slow wave or by a plateau phase of the slow wave. Only few studies have been done on the action of catecholamines, as to the regional difference, the difference between longitudinal and circular muscle layers, or the interaction with prostaglandin production.

1. α-Action. In the *guinea pig* and *rabbit* stomach, stimulation of α-receptors produces both excitatory and inhibitory effects, depending on the portion of the stomach, on the degree of existing mechanical activity, and on the concentration of agonists (19, 152, 156). For example, the circular muscle of *guinea pig* stomach (corpus region) was contracted by low concentrations of epinephrine, but relaxation appeared as the concentration was increased (in the presence of propranolol) (357, 358). The relaxation was mimicked by phenylephrine and antagonized by prazosin or phentolamine, but not by yohimbine or rauwolscine, while the contraction was mimicked by clonidine and antagonized by yohimbine and phentolamine but not by prazosin. Therefore, it was concluded that the relaxation was mediated by α_1- and the contraction by α_2-receptors. However, recently it was found that the contraction caused by epinephrine was strongly inhibited by prazosin, suggesting that activation of α_1-receptors also produces the contraction (S. Chihara and T. Tomita, unpublished observations).

In the circular muscle (longitudinal layer attached) of *guinea pig* stomach, norepinephrine (5 μM) abolished the slow wave and hyperpolarized the membrane, accompanied by a reduction of membrane resistance (263). In the circular muscle obtained from the lower corpus region, epinephrine (up to 100 μM) only transiently reduced the amplitude of slow waves, but the frequency was always increased (about 50% at 100 μM). This effect was mainly mediated through α_1-receptors (S. Chihara and T. Tomita, unpublished observations). The acceleration of rhythmic contractions associated with slow waves appeared already during the hyperpolarization and with a reduction in the amplitude of the slow waves. The transient suppression was followed by an increase in amplitude, shortening of duration, and potentiation of the spike component on top of the slow waves.

In the circular muscle of the antral region of *dog* stomach, the spontaneously generated action potential consisted of a spike and plateau component. Norepinephrine (10 μM), by activating α-receptors, increased the frequency and decreased the amplitude and duration of the plateau, without much change in membrane potential (124). In a few preparations, a clear hyperpolarization (up to 9 mV) was observed.

In the longitudinal strips of *rabbit* stomach, α-receptor stimulation increased ^{42}K efflux irrespective of excitation or inhibition of the mechanical response, suggesting that the increase in K efflux is concomitant with α-receptor stimulation, but that it is not necessarily a link in the chain of events which produce the mechanical response (157, 158).

2. β-Action. Inhibitory effects on both longitudinal and

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circular muscle in the *guinea pig* and *rabbit* stomach are only produced by activation of β-receptors (19, 152, 156). In the methacholine-contracted fundic strip (longitudinal muscle) of *rat* stomach, isoproterenol and fenoterol (β_2-agonist) produced complete relaxation, but prenalterol (β_1-agonist) produced about 50% relaxation, and tazolol (β_1-agonist) had no effect. Both β_1-blocker (practolol) and β_2-blockers ($H_{35/25}$ and ICI 118, 551) shifted the concentration-response curve for isoproterenol to the right. These results suggest that both postjunctional β_1- and β_2-receptors are present in the rat gastric fundus (244).

B. Trachea

The *guinea pig* tracheal muscle produces a high muscle tone, in contrast to the tracheal muscle of most other species (e.g., *dog*, 208, 392; cat, 256). In the *guinea pig* trachea, the muscle tone was abolished by indomethacin or aspirin, suggesting an involvement of prostaglandins in the generation of the tone (46, 326, 327, 356). In isolated *human* trachea and bronchus, a muscle tone also exists. This is insensitive to indomethacin (47, 99, 182), but is suppressed by an antagonist to leukotrienes, FPL 55712 (7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4*H*-1-benzopyran-2-carboxylate) (186). The tone of *guinea pig* as well as *human* tracheal muscles is closely related to a spontaneous rhythmic slow wave activity (180, 380; K. Honda and T. Tomita, unpublished observations). Thus, the β-inhibitory action is easily demonstrated in *guinea pig* and *human* tracheal muscle but, in other species, the tone has generally to be increased by muscarinic agonists or excess K to demonstrate relaxation by β-receptor stimulation.

1. α-Action. Norepinephrine caused contraction, after β-receptor blockade, in the tracheal muscle of *guinea pig*, *rabbit*, *cat*, and old (not young) *rat* (130). *Canine* tracheal and bronchial muscles in situ were contracted by norepinephrine in the presence of propranolol, the response of the bronchus being much stronger than that of the trachea (246, 247). The contractile response of the *dog* and *human* trachea mediated by α-receptors seemed to depend on the basal tone. An increase in the muscle tone by methacholine, histamine, serotonin, or excess K markedly potentiated the response to α-stimulation (22, 220, 325). No evidence for the presence of α-receptors has been obtained in *bovine* (243) and *porcine* tracheal muscles (142).

In the *dog*, intraarterial administration of phenylephrine contracted the trachea after β-receptor blockade, and this was markedly reduced by prazosin, but not by yohimbine (245). Clonidine also produced contraction which was blocked by yohimbine, but not by prazosin. Contraction caused by norepinephrine was partially antagonized by either prazosin or yohimbine. These results indicate that both α_1- and α_2-subtypes are present in this tracheal preparation. However, the contraction mediated by α_2-receptors seems to be predominant. This is in contrast to vascular smooth muscle where the ratio of the two receptor types is reversed, probably related to the paucity of innervation of airway smooth muscle compared with vascular muscle (21–23). Norepinephrine (5 μM), in the presence of propranolol, depolarized the membrane by 8 mV with a slight decrease (10%) in membrane resistance (407). Although it is possible to reveal the presence of an α-excitatory action by blocking β-receptors, the underlying mechanism has not been investigated.

2. β-Action. The original subdivision of β-receptors was based on the finding that the relative potency of a series of catecholamines causing bronchodilation in the *guinea pig* differed from that causing cardiac stimulation in the *rabbit*, and thus, their receptors were termed β_2 and β_1, respectively (234, 235). In later studies, using selective antagonists, it became clear that the *guinea pig* tracheal muscle contained both β_1- and β_2-receptors, although β_2-receptors predominated in both central and peripheral airways (136, 249a, 318, 320, 375, 438, 455). In the trachea, epinephrine and isoproterenol preferentially activated the β_2-type, while norepinephrine activated the β_1-type. The *cat* tracheal muscle contains predominantly β_1-receptors, but the presence of β_2-receptors could be demonstrated (321). In *human* airway muscle, only β_2-receptors were found, and this was considered to be related with the lack of adrenergic innervation (344).

The relaxation of *guinea pig* trachea caused by isoproterenol or epinephrine was shown to be accompanied by suppression of the slow waves and membrane hyperpolarization through activation of β-receptors (probably β_2-subtype) and, under physiological conditions, there was a good correlation between the relaxation and the membrane phenomena measured intracellularly (9, 83, 180, 380). The relaxant action of isoproterenol was not affected by apamin (0.1 μM), TEA (8 mM), or procaine (5 mM), although TEA and procaine abolished the hyperpolarization caused by isoproterenol. The fact that relaxation occurs without hyperpolarization in the presence of TEA or procaine or in excess (40 to 120 mM) K medium shows that hyperpolarization is not a prerequisite for relaxation (9).

In the *guinea pig* (51a, 211, 215a) and *bovine* (428a) tracheal muscles, cholinergic agonists (carbachol, methacholine) antagonized the relaxation caused by β-receptor agonists dose dependently, and this effect was stronger for the relaxation caused by partial agonists, such as soterenol or norfenefrine, than by a full agonist, isoproterenol. In the depolarized condition in 40 mM K medium, isoproterenol was still effective in causing relaxation, but the 50% effective dose (ED_{50}) was increased from 7.2 to 27 nM in the *guinea pig* tracheal muscle (187). When the K concentration was increased to 120 mM, the sensitivity to isoproterenol decreased greatly (9).

In the *bovine* tracheal muscle, studied with the sucrose-

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gap method, it was also found that norepinephrine, isoproterenol, and epinephrine at a concentration of about 5 μM produced hyperpolarization of the membrane and reduced the excitatory junction potential elicited by nerve stimulation (218). Similarly, in the *dog* tracheal muscle, a high concentration of isoproterenol (1 μM) hyperpolarized the membrane by 7 mV without change in electrotonic potential when measured with the microelectrode technique (407). In the later studies, it was found that a lower concentration of isoproterenol (0.5 μM) reduced the resting tension without change in membrane potential and resistance, when measured with the double sucrose-gap method (191). The lack of any effect on the membrane potential was also demonstrated with intracellular microelectrodes; hyperpolarization and a decrease in membrane resistance only appeared when the concentration was increased to 5 μM. In the presence of TEA (5 mM), a spike could be evoked by depolarizing current pulses. Isoproterenol (5 μM) strongly reduced the contraction induced by the spike in the presenceof TEA, but did not affect the spike generation.

In the *dog* tracheal muscle, depolarized by 120 mM K (14.5 mM Na), the relaxation caused by isoproterenol depended on the external Ca concentration (232). In the presence of 10 mM Ca, the relaxation by 10 μM isoproterenol was only 10%, while in 0.1 mM Ca, the relaxation reached 70%. As discussed for the taenia (see section IV), excessive loading of intracellular Ca stores seems to reduce the relaxant effect of isoproterenol.

In the acetylcholine-contracted *dog* tracheal muscle, oxygen consumption was not decreased during the maximal relaxation caused by isoproterenol (10 μM). Therefore, it was considered that energy was required for relaxation, e.g., for the activation of the Ca pump, and that this cancelled out the decrease of energy consumption due to relaxation (231, 232). Although energy expenditure for relaxation due to β-receptor activation would theoretically be expected, this has also not been observed in the *guinea pig* taenia (52a). These results on oxygen consumption of *dog* tracheal muscle are interesting, but should be further analysed.

In the *cat* trachea exposed to Ca-free solution containing 2 mM EGTA, 10 mM caffeine caused a transient contraction probably by releasing Ca from intracellular stores (188). The effect of isoproterenol on the amount of stored Ca could therefore be estimated from the size of the caffeine-induced contraction in Ca-free solution, following a period of Ca readmission. When isoproterenol (0.01 μM) was applied during the loading period with 2.5 mM Ca, the subsequent caffeine contraction (1 min after removal of Ca) was not affected. When acetylcholine (0.1 μM) was applied during the loading period (i.e., in the presence of Ca), it caused a contraction which was reduced if isoproterenol (0.01 μM) was also applied, but the size of the subsequent caffeine contraction (in the absence of Ca) was larger than the control. Thus, the potentiating effect could only be demonstrated if isoproterenol and acetylcholine were applied simultaneously during the loading period, not with isoproterenol alone. It was, therefore, considered that isoproterenol reduces the acetylcholine contraction by facilitating Ca sequestration into an intracellular store which is caffeine sensitive. It seems that, in the *cat* tracheal muscle, this Ca store was nearly saturated under normal conditions (in the absence of acetylcholine) and that isoproterenol acted only when Ca was released from the store (in the presence of acetylcholine).

C. Anococcygeus Muscle

The anococcygeus muscle of most animals is densely innervated by adrenergic nerves, and sympathetic nerve stimulation evokes an excitatory junction potential, as in the vas deferens. Although the excitability appears to be less than that of the vas deferens, this muscle often shows spontaneous activity in several species, especially the *rabbit* (139).

In the pithed *rat*, the contraction of anococcygeus muscle produced by phenylephrine was abolished by prazosin, as expected for α_1-receptors, but the contractions produced by α_2-agonists (clonidine, oxymetazoline, guanabenz, and xylazine) were only moderately antagonized by prazosin. Yohimbine was much less potent than prazosin in reducing the response to phenylephrine, oxymetazoline, and clonidine, but it was as potent as prazosin at reducing the response to guanabenz and xylazine. Thus, in addition to the predominant population of α_1-receptors, postjunctional α_2-receptors are present in this tissue (115). This conclusion was supported by observations on isolated preparations (5, 116). For example, xylazine was a potent agonist, and rauwolscine (α_2-selective) was a more potent antagonist against xylazine than against α-methylnorepinephrine or phenylephrine, although prazosin was more potent than rauwolscine against xylazine (116).

In this muscle, the dose-response curves of phenylethanolamines (phenylephrine, methoxamine) have two components. In contrast, other agonists, such as naphazoline and oxymetazoline, produced a monophasic curve. This has been interpreted by assuming that two different α_1-receptors (α_{1a} and α_{1b}) are present in this muscle, and that the response to high concentrations of phenylethanolamines is mediated by α_{1b}-receptors (282). A similar subdivision of α_1-receptors in the *rat* was made using a derivative of clonidine, Sgd 101/75 (84). This compound produced a maximum response not significantly different from that of norepinephrine, and the same pA_2 value was obtained for phentolamine with both Sgd 101/75 (indanidine) and norepinephrine. However, low concentrations of phenoxybenzamine (0.3 nM) blocked only the response to Sgd 101/75 without much effect on the norepinephrine effect, indicating the presence of different receptors. The effect of phenoxybenzamine was considered not to be due to inhibition of neuronal and nonneuronal uptake of

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norepinephrine, because of the low concentration used. The receptor activated by Sgd 101/75 was designated to be α_{1s}.

A single pulse applied by field stimulation to the *rat* anococcygeus muscle evoked an ejp with a latency of 100 to 250 ms and a contraction, without producing spikes (95). At frequencies higher than 0.5 Hz, a second active depolarization appeared accompanied by a marked increase in tension development. Both electrical and mechanical responses to field stimulation were blocked by phentolamine (0.25 μM). Norepinephrine (0.1 to 30 μM) depolarized the membrane, reduced the membrane resistance, and often produced oscillatory potentials superimposed on the depolarization, particularly during the early phase.

In later studies at room temperature (20–23°C), it was found that field stimulation produced three types of membrane response: a fast excitatory junction potential (ejp) with a latency of less than 100 ms and a time to peak of 300 ms; a slow ejp with a latency of several hundred ms and a time to peak of 1 to 2 s; and an inhibitory junction potential which had a much slower time course. The fast ejp was resistant to prazosin and was mimicked by ionophoretic application of ATP, whereas the slow ejp was mimicked by ionophoretically applied norepinephrine, and these responses were blocked by prazosin (61). These results suggest that the first ejp is caused by ATP and that the slow ejp is caused through α_1-receptor activation by norepinephrine released from the nerve.

When norepinephrine (0.1 to 1 μM) was added to the bath, it depolarized the membrane in two phases: an initial fast phase reaching a peak within 1 to 2 s and a slow sustained phase (62, 242). A low concentration of prazosin (0.01 μM) reduced or abolished both components. When Cl was substituted with benzene-sulfonate, the first component of the depolarization and the norepinephrine-mediated "slow" ejp were abolished, leaving the slow component of the depolarization intact. Thus, the fast phase of the depolarization which corresponds to the "slow" ejp is considered to be due to an increase in Cl conductance of the membrane (62).

In the *rat* anococcygeus muscle, the contraction produced by norepinephrine and by 5-hydroxytryptamine (5-HT) disappeared in Ca-free solution. However, verapamil inhibited only the 5-HT response, not the norepinephrine response. Thus, it was supposed that there are two different Ca channels in this muscle, one being verapamil (and 5-HT) sensitive and the other verapamil insensitive (norepinephrine sensitive) (328). Similar results were obtained with D 600; i.e., the contractions produced by various α_1-agonists were insensitive to D 600, while the responses to excess K were blocked (435). K contractions were tested in the presence of prazosin, or on muscles taken from *rats* pretreated with reserpine, to eliminate the effect of norepinephrine released from nerves. The ineffectiveness of Ca-channel blockers may be explained by assuming that activation of α-receptors opens "receptor-operated Ca channels" which have different properties from those of "voltage-operated Ca channels" which are sensitive to Ca blockers. This will be further discussed in the section on vascular smooth muscles.

In the *mouse* anococcygeus, the order of potency of α-antagonists suppressing the contraction produced by catecholamines was prazosin > phentolamine > yohimbine. This suggested that the postjunctional α-receptors were of the α_1-type. However, the order of potency of agonists was confusing, because it was oxymetazoline > naphazoline > norepinephrine > phenylephrine > methoxamine > xylazine; i.e., the agonist considered to be α_2-selective (oxymetazoline) was much more potent than the α_1-selective agonists (methoxamine and phenylephrine), suggesting the presence of more than one receptor subtype (138). Norepinephrine and Sgd 101/75 had nearly the same potency, but phenoxybenzamine depressed the response to Sgd 101/75 more than that to norepinephrine (85). This was considered to be due to the fact that Sgd 101/75 activates mainly α_{1s}-receptors and norepinephrine both α_{1s}- and typical α_1-receptors, as in the *rat* anococcygeus muscle.

In the *mouse* anococcygeus muscle, both the ejp and the depolarization caused by ionophoretically applied norepinephrine were antagonized by prazosin, not by yohimbine (240). α_2-Agonists (naphazoline and oxymetazoline) also caused depolarization with a long latency, but often produced contraction without depolarization. Since the responses to naphazoline were antagonized by prazosin (0.01 μM), not by yohimbine (1 μM), the possibility was considered that there are two different α_1-receptor subtypes, one being responsible for contraction accompanied by depolarization and another for contraction without depolarization (241).

The innervation of the *rabbit* anococcygeus has a low density compared with the *rat* (94), and it contains homogeneous α_1-receptors (85). Norepinephrine in concentrations higher than 0.3 μM produced a tonic contraction which reached maximum at 300 μM. The contraction evoked by nerve stimulation and that caused by applied norepinephrine were both antagonized by phentolamine. It was often difficult to observe a clear ejp because of the interference by an inhibitory junction potential in response to nerve stimulation (93). The appearance of prominent inhibitory junction potentials is probably related to the high spontaneous activity compared with the *rat* muscle. When the muscle tone was high or raised by histamine, the relaxation caused by isoproterenol was demonstrated to be mediated through activation of β-receptors (94).

D. Summary

The effect of catecholamines on the stomach muscle is complicated in that, in addition to the inhibitory action

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through β-receptors, activation of α-receptors produces both contraction and relaxation, the relaxation being significant particularly when the preexisting muscle tone is high. The contraction seems to be mediated through both α_1- and α_2-receptors, and the relaxation through α_1-receptors. Potentiation of mechanical activity (particularly phasic contraction) is probably related to an increase of spike components appearing on top of the slow wave. The underlying mechanisms of the catecholamine actions are still not known.

In the tracheal muscle, excitatory α-receptors exist, but the mechanisms responsible for contraction have not been analysed. It is clear that the relaxation mediated through β-receptors can occur without change in membrane potential and that some intracellular process, probably mediated by cAMP, is involved in relaxation. This is discussed in detail in section VIII. Under normal conditions, however, it is likely that hyperpolarization of the membrane (and suppression of slow waves in the *guinea pig*) play a causal role facilitating relaxation.

In the anococcygeus muscle, excitation is transmitted through activation of α_1-receptors by norepinephrine released from nerve fibers. A small contribution by ATP as cotransmitter has been found in the *rat*. The receptor type responsible for the response to exogenous catecholamines is mainly the α_1-subtype, but another subtype (α_2- or an atypical α_1-receptor) may also be present at least in the *rat* and *mouse*. The functional significance for these receptor subtypes is not known.

The anococcygeus muscle of *rabbit, dog, cat,* and *ox* contains inhibitory β-receptors, but in the *rat* they seem to be lacking (139). This may be related to the low spontaneous muscle tone in the *rat*. In the *rabbit* anococcygeus, which is highly spontaneously active, a nonadrenergic, noncholinergic inhibitory innervation can be easily demonstrated, but the transmitter has not been identified.

According to studies on *rat* anococcygeus, the depolarization caused by endogenous and exogenous norepinephrine is mainly the result of an increase in Cl conductance of the membrane. The contraction produced by norepinephrine is abolished by Ca removal, but insensitive to verapamil and D 600. The nature of the Ca channels and the mechanism of Ca utilization in this tissue need further investigation.

VII. Vascular Smooth Muscles

The contractility of vascular smooth muscles largely depends on the diameter of the blood vessel and on the proportion of the vessel wall occupied by smooth muscle and by the collagen skeleton. Large blood vessels contract slowly and within a very limited range, while small vessels, particularly in the gut, contract quickly and very strongly and are under strong nervous control. The density of innervation and also the distance between nerve terminals and smooth muscle cells vary considerably (33b). The larger the blood vessel, the greater is the separation, and in some large arteries the innervation only reaches the outer muscle layer, the inner layer not being innervated. On the other hand, small arterioles, in many regions, are densely innervated. The variety of functions is such that the muscle cell membrane of each kind of blood vessel appears to have its own characteristic properties. However, the number of electrophysiological investigations of vascular smooth muscle is as yet very limited. Large vessels are generally very poorly electrically excitable, probably mainly due to the high K permeability of the membrane, while small arteries or arterioles can generate an action potential in response to nerve stimulation and also to exogenous application of catecholamines. Many others have intermediate properties. In this section, we have tentatively divided vascular muscles into small arteries, large arteries, and veins to see whether any correlation can be demonstrated between their functional characteristics and the mechanism of their response to catecholamines. The involvement of intracellular second messengers in the responses to the α- and β-action is discussed in the following two sections VIII and IX.

A. α-Action

Pharmacological analysis of the mechanical response of vascular muscles to exogenous catecholamines and their antagonists has led to the hypothesis that postjunctional α_1-receptors are located at the neuroeffector junction and are activated by norepinephrine released from nerve fibers, whereas α_2-receptors are located extrajunctionally and are activated by epinephrine released from the adrenal medulla or by exogenously applied catecholamines (237, 451, 453). However, in some vascular muscles the situation is more complicated, probably due to the presence of atypical receptor subtypes or a mixture of heterogeneous receptors. Another complication may arise from the contribution by active substances released from the endothelium (15a), as already pointed out in section II. Electrophysiological studies, particularly the recordings of excitatory junction potentials in response to perivascular nerve stimulation, provide some evidence for the existence of a receptor which differs from α_1- and α_2-subtypes.

The contraction of vascular smooth muscles in response to α-receptor activation can be explained by an increase in the intracellular free Ca concentration due to facilitation of Ca influx and/or to intracellular Ca release. The degree of membrane depolarization differs greatly in different muscles, but Ca influx is not necessarily related to depolarization, because Ca can enter the cell through receptor-operated Ca channels which may be voltage insensitive. It is generally considered that there are at least two different Ca pathways in the plasma membrane, one being controlled by the membrane potential (potential-dependent channel) and the other by agonist-receptor interaction (receptor-operated channel) (39, 425). This classification of Ca channels is largely

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based on the susceptibility to Ca channel blockers (see below). It is assumed that activation of the receptor by an agonist increases the probability of the open state of receptor-operated Ca channels. However, the properties of this channel have not been properly analysed. It is possible that, in some contractions, thought to be mediated through receptor-operated Ca channels, a carrier-mediated Ca transport is, at least partly, involved. When Ca influx is mediated by a carrier (or ionophore) system, there may be no increase in membrane conductance, and any changes in membrane potential during Ca influx would then depend on the direction of the net charge movement across the membrane. The question whether Ca influx takes place through different channels or is mediated by ionophores should be clarified. It has been shown, using the patch clamp method, that multiple voltage-dependent Ca channels with different properties exist on the cell membrane in several tissues (280a). Thus, the membrane control of Ca influx and the mode of action of Ca channel blockers on smooth muscle can probably be further analysed in the near future.

The potential-dependent Ca channel seems to be more sensitive to organic Ca channel blockers, such as verapamil, D 600, or nifedipine, than the receptor-operated Ca channel. Thus, Ca channel blockers inhibit strongly the contraction caused by excess K, but only slightly the contraction induced by catecholamines, as demonstrated in the *rat* (140, 277), *rabbit* aorta (285, 364, 427), and *rat* (140) and *rabbit* mesenteric artery (364). However, the sensitivity of the catecholamine response to Ca channel blockers varies significantly in different types of vascular muscle and also depends on the type of receptor activated (283).

Although the low sensitivity of a response to Ca channel blockers may be explained by assuming a large contribution by receptor-operated channels, it is also possible that the properties of Ca channels are modified to various degrees by the surrounding receptor proteins, and as a result of receptor activation. Furthermore, another factor which may affect the apparent relative sensitivity to Ca channel blockers is the contribution of intracellular Ca release to the contraction. One idea which has been put forward is that Ca that is released from intracellular stores not only acts on the contractile machinery but also acts on the receptor-operated Ca channels to reduce their sensitivity to Ca channel blockers (72, 73, 76).

The Ca release mechanism from the intracellular store has not been fully analysed, but Ca-induced Ca release may be partly involved and, as described later (section IX), substances related to phosphatidylinositol may act as intracellular second messengers to release Ca. When Ca release is induced by α-receptor stimulation, refilling of the intracellular Ca pool may occur through a direct pathway from the extracellular space to the pool (66). According to a model proposed by Putney (340a), this pathway is controlled by the Ca concentration in the pool, so that Ca influx to the pool continues as long as Ca is being released by the action of the agonist. On the other hand, sequestration of intracellular Ca into stores is thought to be inhibited during the receptor activation, so that Ca influx is effectively utilized for activation of the contractile machinery (255). These possibilities are very attractive, but need further confirmation. Our understanding of the role of second messengers in the mechanisms of intracellular Ca translocation is as yet incomplete, but recent advances are described in detail in sections VIII and IX.

1. Small arteries and arterioles.

a. *Responses to exogenous catecholamines.*

Mesenteric artery. The contractile response of the *rat* mesenteric artery to exogenous norepinephrine is mediated mainly through α_1 receptors. This conclusion was based on pharmacological analysis, but the additional presence of α_2-receptors is suggested from the slope of the Schild plot and the large pA_2 value for rauwolscine (175). The peculiarity of the receptor is emphasized by the observation that the response to phenylephrine (α_1-agonist) and to naphazoline (α_2-agonist) was antagonized to a very similar extent by either prazosin or yohimbine, but that the slope of the Schild plot was nearly unity for these antagonists. The possibility has therefore been considered that neither agonists nor antagonists may possess a clear selectivity, or that the preparation contains only one class of receptors, close to the α_1-type, but having affinities also to α_2-agonists and antagonists (7, 378). The contraction induced by norepinephrine in the *rat* mesenteric artery consists of a phasic component, which depends primarily on intracellular Ca release, and a tonic component, which depends completely on Ca influx. Prazosin was three orders of magnitude more potent than yohimbine in inhibiting both components (74).

The contraction of *rat* mesenteric artery caused by norepinephrine (1 to 10 μM) was accompanied by membrane depolarization (from −59.2 to −34 mV by 10 μM) (305). However, the norepinephrine contraction was much larger than that induced by excess K which produced the same depolarization. Furthermore, when the membrane was depolarized by excess (40 mM) K, norepinephrine produced contraction without further change in membrane potential. Thus, norepinephrine seems to release Ca from intracellular stores and also to increase Ca influx through a mechanism not directly related to depolarization of the membrane. In this preparation, both the K contracture and the norepinephrine contraction were dependent on external Ca, and they were inhibited by La to a similar extent. On the other hand, nifedipine relaxed the K-contracted artery, but only partially the norepinephrine-contracted artery. Thus, in the artery depolarized by 124 mM K and treated with nifedipine (0.03 μM) to suppress the K contracture,

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norepinephrine (10 μM) could still produce a sustained contraction which was only approximately 20% smaller than the control (174, 379). Similar results were obtained also in the *cat* mesenteric artery (379).

The *guinea pig* mesenteric artery was depolarized by clonidine (10 μM), and this was blocked by prazosin (1 μM), but not by yohimbine (1 μM), suggesting activation of α_1-receptors for the depolarization (195). The contractions induced by norepinephrine were much larger than those induced by excess K which depolarized the membrane to the same degree as norepinephrine (1 to 10 μM) (41). The membrane resistance was decreased by norepinephrine at concentrations which depolarized the membrane by more than 5 mV, but this was mainly a secondary effect due to the depolarization. When the membrane was depolarized by more than 12 mV, the decrease in membrane resistance by norepinephrine was greater than that observed during depolarization by passing current only.

In the *dog* mesenteric artery, the membrane was depolarized by norepinephrine at concentrations higher than 0.03 μM, and electrical slow waves were induced with concentrations higher than 0.1 μM. These effects were antagonized markedly by yohimbine (1 μM), but only weakly by prazosin (1 μM), suggesting that mainly α_2-receptors contributed to the depolarization (403). This is interesting because the receptors responsible for contraction produced by phenylephrine and naphazoline are reported to be atypical α_1-receptors similar to those in the *rat* artery and very effectively antagonized by prazosin (7). This suggests that the contraction is not linked with membrane depolarization. Furthermore, there seems to be a species difference in the receptor type responsible for the depolarization between *dog* and *guinea pig*.

In the *rabbit* mesenteric artery, the contraction caused by norepinephrine (0.5 to 12 μM) was blocked by prazosin (0.3 μM), not by yohimbine (3 μM) (230), and the membrane depolarization caused by norepinephrine (0.1 to 10 μM) was also suppressed by prazosin (1 μM) (196, 297). The maximum depolarization by norepinephrine (100 μM) was completely blocked by 10 μM diltiazem, whereas that induced by 80 mM K was unaltered, suggesting that an increase in Ca conductance contributes to the depolarization (73). In the main part of the *rabbit* mesenteric artery, the K contracture was more sensitive to Ca channel blockers than the norepinephrine contraction (49). But in the peripheral part of mesenteric artery, the contraction induced by norepinephrine (10 μM) was more sensitive to diltiazem than the K contracture (75). This part has a 10^4-fold higher sensitivity to diltiazem than the aorta, probably due to the fact that the norepinephrine contraction in the peripheral part of the artery is nearly entirely dependent on Ca influx (76), in contrast to the main part of the artery where intracellular Ca release is also involved.

In the *rabbit* mesenteric artery exposed to Ca-free (bicarbonate) buffer containing 2 mM EGTA and 2.5 mM Mg, the contractions evoked by norepinephrine (10 μM) applied every 5 min for 1 min decreased successively and disappeared after the fourth application (196). On the other hand, similar applications of caffeine (10 mM) produced a contraction only once or twice. Norepinephrine failed to produce a contraction after caffeine application, while caffeine could produce a small contraction after the norepinephrine-evoked contraction had ceased. The results were interpreted as indicating that Ca is partly reaccumulated into the intracellular store after the norepinephrine contraction, but that Ca is effectively extruded from the cell after the caffeine contraction. In other experiments, in which Ca-free solution [N-(2-hydroxyethyl)-1-piperazine-N-2-ethanesulphonic acid (HEPES buffer)] containing 2 mM EGTA and 0 mM Mg was used, norepinephrine (10 μM) produced a transient contraction only once, a second application being ineffective. On the other hand, 25 mM caffeine, given after the norepinephrine response, was still able to produce a large contraction. Thus, the norepinephrine-sensitive Ca store seems to be different from the caffeine-sensitive store. The possibility has been considered that Ca is released from the norepinephrine-sensitive store, located at the plasma membrane, and triggers in turn Ca release from the caffeine-sensitive store which is probably the sarcoplasmic reticulum (360).

Splenic artery. In the *dog* splenic artery, the presence of a single population of α-receptors was shown which had characteristics of both α_1- and α_2-types, judging from the Schild analysis using prazosin and rauwolscine (165), but this receptor was much closer to the α_1-type (102). In this artery, verapamil (0.5 to 50 μM) reduced the contraction caused by norepinephrine and methoxamine at concentrations of 2.5 to 13 μM (102), but it is possible that this effect of verapamil in high concentrations is partly due to α-receptor blocking action (36a).

Renal artery. In the *guinea pig* renal artery, norepinephrine (0.5 μM) or phenylephrine (0.5 μM) produced contraction accompanied by depolarization of the membrane (about 5 mV) and reduction of the membrane resistance (about 10%). These responses were blocked by prazosin (0.1 μM), not by yohimbine (266).

Coronary artery. In the *dog* large coronary (left circumflex) artery, studied on helical strips, α_1-receptors are dominant (345), while in the small coronary artery (resistance vessels), monitored by coronary flow, α_2-receptors are dominant (178).

Although most vascular muscles can produce a transient contraction in response to α-receptor activation at least once in Ca-free solution, the *dog* large coronary artery, which has predominantly α_1-receptors, is very sensitive to Ca removal. The contractile response to 10 μM norepinephrine or 10 μM cirazoline (α_1-selective agonist) was almost completely blocked by a 5- to 10-min

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exposure to Ca-free solution containing 2 mM EGTA (345, 428). A similar suppression was observed by adding 10 mM La or organic Ca channel blockers (verapamil, D 600, nimodipine, diltiazem, or SK & F 525A (proadifen)) in the presence of Ca. Thus, the large coronary artery seems to rely nearly exclusively on the influx of external Ca for its contraction. The *dog* circumflex coronary artery contracted upon stimulation of either α_1- or α_2-receptors, when examined with α_1- (phenylephrine) and α_2- (guanfacine) agonists, and with α_1- (corynanthine and prazosin) and α_2- (rauwolscine and yohimbine) antagonists. Nifedipine was equally potent in inhibiting both the responses to α_1- and α_2-receptor activation (304).

Cerebral artery. The *cat* middle cerebral artery has predominantly α_2-receptors (379). In this muscle, the contractile response to norepinephrine (10 μM) was quickly abolished by removal of Ca. On the other hand, in the *rat* middle cerebral artery, which has mainly α_1-receptors, the early part of the contractile response to norepinephrine was resistant to Ca removal, indicating that it was partly due to intracellular Ca release.

Saphenous artery. In the *rabbit* saphenous artery, a low concentration of norepinephrine (lower than 5 μM) produced contraction without depolarizing the membrane. At higher than 5 μM, the membrane was depolarized by norepinephrine dose dependently (177). This depolarization was not blocked by phentolamine, phenoxybenzamine, or prazosin, although these blockers completely abolished norepinephrine-induced contractions. In the *dog* saphenous artery, nifedipine blocked the contraction caused by guanfacine (α_2-agonist), but had a weak inhibitory effect on that caused by phenylephrine (α_1-agonist) (304).

Ear artery. The *rabbit* ear artery contains only typical α_1-receptors, according to studies with selective antagonists (prazosin and rauwolscine) (165). It was shown that there was a good correlation between membrane depolarization and tension generated by norepinephrine (0.1 to 10 μM) (421). In other reports, it was observed that norepinephrine at low concentrations (between 5 nM and 1 μM) caused contraction without modifying the membrane potential, although the membrane was depolarized at high concentrations (121, 177). The α-antagonists did not suppress the depolarization, whereas they abolished the norepinephrine-induced contraction, as in the saphenous artery (177). In spite of the insensitivity of the membrane potential, an increased permeability to K, Na, and Cl ions was suggested from the effects of norepinephrine on the ion fluxes (121).

In the *rabbit* ear artery perfused at a constant flow, extra- and intraluminally, the pressure response to norepinephrine and phenylephrine was measured. The dependence of the response on external Ca suggested that activation of α_1-receptors mobilized both extra- and intracellular Ca, which was suppressed by prazosin (0.03 to 0.3 μM), but not by yohimbine (3 μM) (268). The external Ca-dependent contraction produced by norepinephrine (5 μM) was concentration dependently inhibited by nifedipine (10 to 300 nM) and verapamil (1 to 50 μM), while the contraction due to mobilization of intracellular Ca was not affected by nifedipine (300 nM), but slightly (by 20%) inhibited by verapamil (50 μM) (265). The size of contraction of a helical strip, induced by norepinephrine (1 μM), decreased roughly exponentially with the time of exposure to Ca-free solution containing 2 mM EGTA, the half-time being about 35 min (121). The amount of ^{45}Ca which can be released by norepinephrine also decreased with a similar time course after Ca removal. Two different pathways are proposed for filling the norepinephrine-sensitive store with external Ca (66). One is a pathway in the plasma membrane through which Ca passes into the cytoplasm and is then taken up by the store, and the other is a direct pathway through which Ca penetrates into the store, without passing through the cytoplasm, i.e., without causing contraction. The direct filling process during readmission of external Ca was blocked by Mn or La, but was not affected by organic Ca channel blockers, such as D 600 or nifedipine, as observed for the *rabbit* aorta by Karaki et al. (209; see section on large arteries).

The *guinea pig* ear artery was depolarized by norepinephrine at concentrations higher than 0.1 μM, and this was accompanied by a decrease in membrane conductance (207).

Tail artery. In the *rat* tail artery, using prazosin and corynanthine as α_1-antagonists and idazoxan as an α_2-antagonist, it was demonstrated that exogenous norepinephrine activates predominantly α_1-receptors and partly α_2-receptors. On the other hand, since 1 nM prazosin reduced the response to nerve stimulation (1 to 5 Hz) by over 80% and since 100 nM virtually abolished the response, it was concluded that only α_1-receptors are involved in the response to norepinephrine released by sympathetic stimulation (284). In the *rat* tail artery, the response to the low concentration of norepinephrine applied to the bath was essentially the same as that seen in the *rabbit* ear artery; i.e., the contraction occurred without depolarization of the membrane (177).

b. Electrical responses to nerve stimulation. In small arteries, stimulation of perivascular nerves produces an ejp, and this is often followed by a slow depolarization, particularly with high frequency repetitive stimulation. The ejp is due to activation of junctional receptors by the transmitter (norepinephrine or possibly some other substance). The slow depolarization is considered to be due to norepinephrine released from nerve terminals, diffusing beyond the junctional membrane area, and activating α-receptors situated in the extrajunctional area of the membrane. In large arteries, such as the *rabbit* carotid artery, where nerve terminals and muscle cells are widely separated, nerve stimulation does not evoke a

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discrete ejp, but repetitive stimulation at high frequencies causes only a slow depolarization of the membrane (291).

Submucosal arteriole. In the submucosal arterioles (15- to 150-μm inner diameter) of *cat* stomach, the ejps were not suppressed by phentolamine (3 μM), probably due to block of α_2-receptors located on nerve fibers which would result in an increase of the amount of norepinephrine released from the nerve. However, the ejps were reduced by 82% by prazosin (3 μM) in 30 min (301). Norepinephrine and phenylephrine (1 to 100 μM), exogenously applied, clearly depolarized the membrane and often initiated oscillatory potentials or spikes, while clonidine (10 to 300 μM) produced no significant depolarization. The phenylephrine contraction was larger than that caused by clonidine. When concentrations of phenylephrine were adjusted to produce the same mechanical response as clonidine, the depolarization was 5 to 9.8 mV with phenylephrine and less than 0.5 mV with clonidine. The responses to phenylephrine were antagonized by prazosin (1 μM), and those to clonidine were antagonized by yohimbine (1 μM). Therefore, it seems that α_1-receptors mediate the "electromechanical" coupling (the ejp by endogenous and the depolarization by exogenous norepinephrine), while α_2-receptors mediate "pharmacomechanical" coupling. The electro- and pharmacomechanical coupling will be described further in the section on large arteries.

The properties of submucosal arterioles (25- to 60-μm diameter) of the *guinea pig* small intestine seem to differ from those of *cat* stomach. In these arterioles, the ejp was not affected by α-blockers (phentolamine, tolazoline, prazosin, or labetalol, at 2.5 to 5 μM) (168). The most common response to iontophoretic application of norepinephrine with a microelectrode was a localized contraction without change in membrane potential, and this response was abolished by phentolamine. Occasionally, at some particular spot of the arteriole, norepinephrine produced a depolarization, similar to the ejp, without contraction, even in the presence of phentolamine (170). These results have been interpreted to indicate that the receptor at the junctional spot close to nerve fibers has different properties from those of the α-receptor located in the extrajunctional area. Thus, a third subtype, resistant to α-blockers, has been proposed and called "γ-receptor" (169, 171, 310), similar to the idea of the "junctional" receptor proposed for the *guinea pig* vas deferens (181).

Basilar artery. The *rat* basilar artery does not contract in response to exogenous norepinephrine (171, 175). This artery was very insensitive to norepinephrine, which caused a very slow depolarization only at high concentrations (more than 200 μM), and the depolarization was not affected by either prazosin, phentolamine, or propranolol. A similar insensitivity to norepinephrine has been reported in the *guinea pig* basilar artery (210), but the *cat* basilar artery was depolarized and contracted by norepinephrine (0.03 to 1 μM) (159). External field stimulation evoked ejps in the *rat* basilar artery, presumably due to norepinephrine release, but since they were also resistant to adrenergic blocking agents, it was thought that the "γ-receptor" might be responsible for the neuroeffector transmission (171), as in the submucosal arteriole (169). In this artery, local application of norepinephrine close to the preparation, but not bath application, evoked a fast depolarization, possibly by activating so-called γ-receptors (60).

It has been argued that the failure to block the ejps by α-blockers might be due to the extremely high concentration of transmitter at the junctional cleft (33), or to another transmitter, different from norepinephrine, for example ATP, as described in the section on the vas deferens (281). Iontophoretic application of norepinephrine may stimulate nerve terminals and may result in the release of some yet unidentified transmitter. Further demonstration of the existence of "γ-receptors" in many other tissues will be necessary before the concept of a third type of catecholamine receptor is finally accepted.

In the *dog* basilar artery, perivascular nerve stimulation produced an initial transient contraction followed by a relaxation, or a late slow contraction, or a transient relaxation and slow contraction (307). Electrical stimulation increased the release of [^{3}H]-norepinephrine or [^{3}H]purine compounds, after preincubation with these substances. When exogenously applied, norepinephrine (10 to 100 μM) produced a slow contraction, while ATP (0.5 to 3 μM) produced a transient contraction followed by a slow relaxation, similar to electrical stimulation. These results suggest that, in this muscle, ATP may act as a transmitter in addition to norepinephrine.

Ear artery. In the *rabbit* ear artery, perivascular stimulation evoked ejps. An all-or-none action potential was initiated following summation of ejps at frequencies higher than 1 Hz (177, 400). Contraction was associated with action potentials. The ejps and the neurally evoked contractions were insensitive to phenoxybenzamine, prazosin, or labetalol, even at 25 to 30 μM, but phentolamine (3 to 30 μM) depressed neurally evoked contractions.

In other experiments in which only the mechanical response was studied (332), it was also found that phentolamine (1 μM) blocked the response to stimulation of perivascular nerves at 2 Hz and decreased it by at least 90% at 4 and 8 Hz. However, since phentolamine also depressed the contraction produced by direct muscle stimulation, the possibility was considered that it interferes with some step in excitation-contraction coupling, rather than blocking the receptors (177).

In later studies, it was noted that a slow depolarization followed the ejp evoked by nerve stimulation of the *rabbit* ear artery (405). This depolarization was suppressed by prazosin (1 μM) without affecting ejps. The contraction mediated by nerve stimulation (10 Hz) was reduced to

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49% of the control by prazosin (1 μM). Norepinephrine application depolarized the membrane and produced contraction, and both responses were also antagonized by prazosin. At high frequencies (>5 Hz), a spike was evoked on top of a facilitated ejp, the spike being blocked by nicardipine (a Ca channel blocker, 0.1 μM). In the presence of prazosin and nicardipine, the contraction evoked by perivascular nerve stimulation was reduced to 34% of the control. Since the slow depolarization and spike activity were blocked by these drugs, the remaining ejp seemed to produce some mechanical response. This is different from the observation on the arteriole of *guinea pig* intestine, in which ejps did not evoke contraction (168).

Recently it was shown with the method of iontophoretic application that ATP produced a rapid depolarization similar to the ejp, while norepinephrine produced only a slow depolarization (404). However, further evidence has to be presented before ATP is accepted to be a transmitter in this artery.

Tail artery. In the *rat* tail artery, nerve stimulation evoked ejps and a slow depolarization which were not affected by yohimbine (130 μM) (79). Prazosin (12 nM) abolished only the slow depolarization without affecting the ejp (80), as in the *rabbit* ear artery. In contrast, in the *rat* tail artery, the slow depolarization caused by nerve stimulation and, also, the depolarization by norepinephrine application were not blocked by prazosin (1 μM), but reversibly antagonized by yohimbine (0.5 μM), although the contraction induced by norepinephrine was more effectively suppressed by prazosin than yohimbine (195). The reason for the different results is not clear.

The mechanical response to a single stimulus of perivascular nerves in the *rat* tail artery consisted of two components, an initial small contraction due to a muscle action potential and a second slow contraction due to depolarization by extrajunctional α-receptor activation (309). A weak single stimulus produced only an ejp and no tension response. As the stimulus intensity was increased, some active electrical response (graded action potential) appeared, and this was accompanied by tension development. The contraction caused by a train of stimuli was mainly due to α-receptor activation (which was blocked by prazosin, 1 μM), even when an action potential was evoked by the ejp in response to each stimulus. Thus, the contribution of the action potential to the muscle contraction seems rather small. The physiological significance of the ejp and of the generation of action potentials for the muscle contraction needs further clarification.

There is some evidence in the *rat* tail artery that the transmitter for the ejp is ATP, while that for the slow depolarization is norepinephrine, because the former was selectively suppressed by α,β-methylene ATP (1 μM), a stable analogue of ATP, and the latter was blocked by phentolamine (2 μM) (384). This agrees with the finding in the *guinea pig* vas deferens (383, 385).

Saphenous artery. The properties of the ejp and the response to exogenously applied norepinephrine in the *rabbit* saphenous artery were essentially similar to those in the *rabbit* ear and *rat* tail arteries (177).

Mesenteric artery. In the *guinea pig* mesenteric artery, perivascular nerve stimulation produced an ejp without a slow depolarization (195). Prazosin (0.1 μM) failed to modify ejps, but suppressed the contraction evoked by nerve stimulation. Thus, also in the mesenteric artery, the ejp and contraction are not closely linked.

In the *rabbit* mesenteric artery, in addition to ejps, a slow depolarization was observed with high frequency stimulation (over 5 Hz) (297). The ejp was not suppressed by α-blockers, but the slow depolarization was blocked by prazosin. As in the tail artery, ATP may be responsible for the ejp, because there is some evidence that ATP and norepinephrine act as cotransmitters (230, 306). ATP (3 to 1000 μM) applied extraluminally produced vasoconstriction. The nerve-mediated constriction was slightly reduced either by α-blockers or by α,β-methylene ATP (3 to 15 μM) and, in the presence of α,β-methylene ATP, the contraction was abolished by phentolamine or prazosin. Supporting evidence for involvement of ATP in the ejp was also obtained by studying the suppressing effect of α,β-methylene ATP (0.1 to 1 μM) on ejps (185).

Renal artery. In the *guinea pig* renal artery, a single stimulus applied to perivascular nerves did not evoke any response, but repetitive stimulation (5 pulses, 50 Hz) produced a small transient depolarization (less than 2 mV). This response was resistant to α-blockers. Prazosin produced a weak suppression only at higher concentrations over 10 μM, although it completely blocked the norepinephrine-induced depolarization at 0.1 μM (266).

2. Large arteries.

Aorta. In the *dog, guinea pig*, and *rabbit* aorta, the predominant receptor is the α_1-type (113, 148, 337, 352, 354). The α_1-selective agonists (phenylephrine, methoxamine) and antagonist (prazosin) are relatively potent in most species, while the sensitivity to α_2-agonists and antagonists differs in different species. The *rabbit* and *guinea pig* aortae were shown to be least sensitive to clonidine and yohimbine, while the *hamster, cat*, and *dog* aortae were intermediate, and the *rat* aorta was highly sensitive to both drugs (118, 119, 351). There is general agreement that the *rat* aorta contains α_1-receptors, because of the high affinity for prazosin (111, 260, 341, 354) and because corynanthine was much stronger than rauwolscine in antagonizing α_1- and α_2-agonists (112). However, clonidine (α_2-agonist) is also very effective in producing contractions (355). Since the contraction produced by clonidine is more effectively antagonized by prazosin than by yohimbine, it is considered that the *rat* aorta contains, in addition to α_1-receptors, another type of receptors which cannot be simply classified as α_1- or

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α_2-type (341), or that it is a receptor with both α_1- and α_2-properties (352, 354).

In the *rabbit* aorta, the contraction caused by 10 μM norepinephrine decreased during exposure to Ca-free (Mg-free) solution. After 15-min exposure, the remaining tension development was dependent on the concentration of EDTA added to the solution. The response nearly disappeared with 1.5 mM EDTA. Since EDTA is mainly confined to the extracellular space, the slowly depleted Ca stores responsible for the norepinephrine response were considered to be located at relatively superficial membrane sites (446). A recent study of ^{45}Ca uptake indicated also that the Ca supply for the increased Ca influx by 10 μM norepinephrine came from two different sources (252). The results suggested that the Ca source responsible for the initial phase of the contraction is a pool bound at the plasma membrane, and that for the maintained phase it is the free Ca in the extracellular space. The contraction induced by norepinephrine (1 μM) in Ca-free medium at pH 7.3 was decreased after pretreatment with a medium at pH 5.1, and the norepinephrine-sensitive ^{45}Ca efflux disappeared. Since the microsomes prepared from the *rabbit* aorta had high affinity binding sites for Ca, which were reduced at low pH, the possibility has been considered that pH-sensitive Ca-binding sites at the plasma membrane are the norepinephrine-sensitive Ca pool (149).

The initial fast phase of the norepinephrine contraction was only slightly reduced by 2 mM La or Ca removal (with 10 mM EGTA, 10- to 15-min treatment), but the slow maintained phase of the contraction was strongly depressed (108). Similar results were obtained with phenylephrine as an agonist (91). Thus, for the early phase of the contraction, in addition to Ca influx, intracellular Ca is likely to be utilized. In the presence of La or EGTA, a second application of norepinephrine failed to evoke a contraction. There seems to be no reuptake of intracellular Ca into the norepinephrine-sensitive store which is likely to be different from the store responsible for Ca uptake during relaxation (108). Prazosin was three orders of magnitude more potent than yohimbine in inhibiting the increase of ^{45}Ca influx by norepinephrine in the presence of Ca, and also inhibiting the contraction in Ca-free medium (17, 71). The same results were obtained with *rat* aorta (74). Thus, in these preparations, α_1-receptors are responsible for both Ca influx and Ca release. The norepinephrine-sensitive Ca store was gradually depleted with a half-time of 12 min or 24 min in Ca-free (1 mM EGTA) solutions containing bicarbonate or HEPES buffer, respectively (209). Readmission of Ca quickly refilled the store, but the Ca loading was greatly reduced by pretreatment with La (0.1 to 0.5 mM), not with verapamil or D 600 at a concentration of 1 μM.

In the *rabbit* aorta, both norepinephrine and caffeine produced a transient contraction and increased ^{45}Ca efflux when applied 10 min after removal of external Ca. A prior application of caffeine depleted the norepinephrine-releasable Ca store, suggesting that norepinephrine and caffeine release Ca from a common source, likely to be the SR (107). The amount of Ca in the store was studied by observing contraction and ^{45}Ca efflux in Ca-free medium and was estimated to be 74 μmol/kg wet weight under normal conditions. The concentration of Ca was calculated to be 5.3 mM, assuming that smooth muscle cells occupy 40% of the muscle weight and that the SR occupies 3.5% of the cell volume (249). This amount decreased exponentially with a half-time of 34 min after Ca removal at 37°C.

A Ca channel blocker, cinnarizine (10 μM), had no effect on the response to norepinephrine (10 μM), although it strongly inhibited the K contracture (49). Similarly, the Ca-induced contracture in excess K solution was noncompetitively inhibited by diltiazem, and this inhibition was parallel to the blockade of ^{45}Ca influx. Diltiazem markedly suppressed the contraction induced by a low concentration of norepinephrine (0.01 μM), but less effectively that induced by high concentrations (1 to 10 μM), probably due to a significant contribution by intracellular Ca release (426). Ca released intracellularly may reduce the sensitivity of the Ca channels to Ca channel blockers at the plasma membrane (72, 73, 76).

In the *rat* aorta, contractions produced by phenylephrine, cirazoline (α_1-selective), or norepinephrine were only weakly suppressed by D 600 or cinnarizine, whereas contractions produced by partial agonists, St 587 (2-(2-chloro-5-trifluoromethylphenylimino)imidazoline), Sgd 101/75 (α_2-selective), clonidine, or oxymetazoline (α_2-selective) were very markedly inhibited by the Ca channel blockers at concentrations of 1 to 10 μM (30, 312). The contractions caused by these agonists were all very effectively antagonized by prazosin. Similar results were obtained by removing the external Ca; i.e., omission of Ca resulted in a greater reduction of the response to clonidine or oxymetazoline than that to norepinephrine or phenylephrine (141, 313). Thus, the α-receptors in *rat* aorta seem to contain two different subtypes, both of which have pharmacological properties similar to the α_1-subtype, but their sensitivity to Ca channel blockers and Ca removal is different. However, when interpreting such data, it has to be borne in mind that the response to partial agonists may be more susceptible to block than that to full agonists (201a).

More detailed observations have also been reported. The contractions due to activation of α_1-receptors by phenylephrine, as well as those due to activation of α_2-receptors by clonidine, consisted of an initial fast component and a slow component. The fast component caused by norepinephrine (1 μM) or phenylephrine (10 μM) was somewhat larger (74%) than that caused by clonidine (10 μM) (43%), probably reflecting a contribution by intracellular Ca release to α_1-receptor activation. The slow component was selectively suppressed by nife-

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dipine, independent of the type of receptors activated (362).

In contrast to the observation on *rat* aorta, the contractions of the *guinea pig* aorta produced by St 587, Sgd 101/75 (α_1-selective), as well as clonidine (α_2-selective) were not much affected by D 600 (30). Pharmacological analysis indicated that these agonists stimulate typical α_1-receptors. Thus, the *guinea pig* aorta contains only a single type of α_1-receptors which mediate the contraction that is resistant to Ca channel blockers.

Carotid artery. In the *sheep* carotid artery, the inner (noninnervated) muscle layer was more sensitive to norepinephrine than the outer (innervated) layer, even in the presence of desipramine, which prevents uptake of norepinephrine by the nerves (146, 215). However, nerve activation by nicotine (10 μM) produced roughly similar degrees of contraction in both layers, indicating an efficient nervous control of the inner layer (215). When the pressure throughout the vessel wall was raised by placing a cylindrical clip around the artery *in vivo* for more than 10 days, the nerve fibers disappeared (146). This suggested that the lack of innervation of the inner muscle layer may be due to the high pressure.

In the *rabbit* carotid artery, a difference was found between the responses of the outer and inner muscle layers to nerve stimulation and to exogenously applied norepinephrine (291). The muscle fibers in the outer, innervated layer produced no clear ejps in response to nerve stimulation at low frequency, but at high frequency (above 20 Hz), they produced a slow depolarization. However, a contraction without depolarization was observed at frequencies below 5 Hz. The muscle fibers in the inner, noninnervated layer were depolarized by norepinephrine (5 to 8 mV at 1 μM, 12 to 15 mV at 10 μM), whereas those in the outer layer were less sensitive (no significant depolarization below 10 μM, 4 to 8 mV at 50 μM, 6 to 12 mV at 200 μM).

The depolarization caused by epinephrine was accompanied by a reduction of membrane resistance (292). In some preparations, epinephrine (1 μM) produced oscillatory electrical activity. Substitution of Cl with sulphate, NaCl with sucrose, or Na with choline all reduced the effects of epinephrine on the membrane, suggesting that a change in Na and Cl conductances contributed to the depolarization.

Pulmonary artery. The *rabbit* and *dog* pulmonary arteries contain postjunctional α_1-receptors (88, 116, 390). However, the *rabbit* pulmonary artery was shown to be also sensitive to clonidine (α_2-agonist), but the response to clonidine was more effectively antagonized not only by yohimbine but also by prazosin than the response to methoxamine (α_1-agonist). These results suggest that this artery may contain atypical α_1-receptors or some receptors similar to the α_2-type which are sensitive to prazosin, in addition to α_1-receptors (176). In the *rabbit* main pulmonary artery, the contraction evoked by nerve stimulation was suppressed by corynanthine (α_1-antagonist), and a nearly complete block was obtained with 10 μM (445). As in the aorta, the contraction of the *rabbit* main pulmonary artery caused by methoxamine was only weakly reduced while the clonidine contraction was markedly reduced by verapamil and also by removal of external Ca (176).

In the *rabbit* main pulmonary artery, low concentrations (0.02 to 0.1 μM) of norepinephrine elicited mechanical responses but no depolarization of the membrane when measured with microelectrodes inserted from either the intimal surface (396) or the adventitial surface (69). The process which underlies a contraction without depolarization has been termed "pharmacomechanical coupling" (387, 389). However, at high concentrations (5 μM), norepinephrine depolarized (9 mV) and elicited a larger contraction (69). When the microelectrode was inserted from the endothelial side, a dose-dependent depolarization (from −60 mV to −43- to −45 mV) was observed with norepinephrine (0.01 to 1 μM) or with methoxamine (0.3 to 10 μM), associated with an increase in tension. The depolarization reached a more or less steady level at 1 μM norepinephrine, but the tension increased further with higher concentrations (153, 154). In the presence of 10 mM TEA, which decreased the membrane potential roughly from −60 to −50 mV, the depolarization by norepinephrine was potentiated. Since, in the *rabbit* carotid artery (see above), the inner layer was depolarized by lower concentrations of norepinephrine than the outer layer (301), such differences between the properties of the muscle layers may be one of the reasons for the discrepancy concerning the change of membrane potential reported by Casteels et al. (69) and by Haeusler (153, 154).

The membrane resistance was decreased by methoxamine dose dependently over the entire range of the concentration-contraction curve (154). These catecholamine effects were blocked by prazosin, suggesting a main contribution by α_1-receptors. The norepinephrine contraction was markedly increased by TEA. Presumably, in the absence of TEA, the increase of intracellular Ca by norepinephrine activates the K conductance, thereby counteracting the depolarization, but in the presence of TEA, this counteraction is suppressed, because the increase in K conductance is blocked (155).

When NaCl was replaced with sucrose, the depolarization by norepinephrine disappeared, and the contraction was markedly decreased (69). In addition to its contribution to the norepinephrine-induced depolarization, Na may influence the pharmacomechanical coupling. The slow sustained contraction caused by low concentrations of norepinephrine, without depolarization, was dependent on the presence of external Ca, suggesting that Ca influx is responsible for the contraction. On the other hand, high concentrations (above 0.25 μM) of norepinephrine produced a phasic contraction in

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Ca-free solution, probably by causing Ca release from cellular stores.

Using electron probe X-ray microanalysis, it was shown in the *rabbit* pulmonary artery that the Ca content in the sarcoplasmic reticulum was reduced during the norepinephrine contraction (223), as observed for the *guinea pig* portal vein (42).

The *guinea pig* pulmonary artery was depolarized by norepinephrine at concentrations (above 0.05 μM) lower than those effective in the *rabbit* pulmonary artery (402). The depolarization reached maximum at 0.25 μM which depolarized the membrane from about −50 to −40 mV. When the concentration was increased to more than 1 μM, action potentials were elicited, superimposed on the sustained depolarization.

Perivascular nerve stimulation produced an ejp with a very slow time course in the *guinea pig* main pulmonary artery. The time to reach the peak was 10 to 15 s after stimulation, and the falling phase lasted 2 to 3 min. The depolarization occasionally evoked a spike during the rising phase. Phentolamine (1 μM) blocked the spike and the ejp, as well as the electrical response to exogenous norepinephrine 15 min after application. Phenoxybenzamine (0.5 μM) and prazosin (1 μM) had a similar blocking action (402).

3. Potassium efflux in arteries.

Catecholamines affect the K permeability of the membrane; i.e., they may directly reduce the K conductance leading to membrane depolarization. However, it is also possible that they may secondarily increase the K permeability as a result of membrane depolarization, or through an increase of the intracellular Ca concentration (by way of the Ca-activated K conductance).

Norepinephrine (10 to 100 μM) was found to increase K efflux in a number of arteries (studied with ^{86}Rb). The increase of the efflux in *rabbit* ear artery and aorta and in *rabbit* and *guinea pig* pulmonary artery (high fluxing arteries) was larger than that in *rabbit* and *guinea pig* mesenteric artery, *rabbit* brachial artery, and *guinea pig* abdominal aorta (low fluxing arteries) (40). There was no correlation between ^{86}Rb efflux and the degree of membrane depolarization. Prazosin (0.1 μM) produced roughly a 10-fold shift of the dose-tension curve for norepinephrine in both *rabbit* aorta (high fluxing) and mesenteric artery (low fluxing), and it reduced the norepinephrine-induced ^{86}Rb efflux and contraction to a similar extent in both arteries. Thus, the difference in ^{86}Rb efflux in these arteries does not appear to be related to the difference in receptor subtype. In the *guinea pig* pulmonary artery, the depolarization, the contraction, and the increase in ^{86}Rb efflux during activation of α-receptors by epinephrine (at 23°C) were not affected by apamin (0.3 μM) which blocked the epinephrine response in the *guinea pig* taenia, suggesting that different mechanisms are involved in the increase in K conductance in these tissues (105).

In the *rabbit* aorta, the increase of ^{86}Rb or ^{42}K efflux caused by norepinephrine (10 μM) was significantly greater in Ca-free medium containing 0.1 to 0.2 mM EGTA than in the presence of Ca, suggesting that the K channels opened by α-receptor activation differ from Ca-activated K channels (40). This conclusion is supported by the observation that TEA (10 to 20 mM) reduced substantially the ^{86}Rb efflux evoked by excess K, whereas that evoked by norepinephrine was only slightly reduced.

The contraction of the *rabbit* aorta caused by norepinephrine (0.01 to 10 μM) was accompanied by a parallel increase in ^{86}Rb efflux (275). However, dissociation of these changes was observed in the presence of verapamil and La. Verapamil (10 μM) reduced the contractile response only 12%, but the increase in ^{86}Rb efflux by 65%. In the presence of 1.3 mM La, norepinephrine (1 μM) still produced a significant contraction, although only once, without any concomitant increase in ^{86}Rb efflux. This suggests the possibility that ^{86}Rb efflux is increased as a result of Ca influx, not by intracellularly released Ca. However, in recent studies on ^{42}K efflux in the *rabbit* aorta, the increase of the rate constant for ^{42}K efflux and of the mechanical response caused by a low concentration of norepinephrine (0.1 μM) was abolished by diltiazem (10 μM), but that caused by a high concentration of norepinephrine (10 μM) was only slightly diminished (1). This indicates that intracellularly released Ca is also able to increase ^{42}K efflux. Furthermore, the increase of the rate constant for ^{42}K efflux caused by norepinephrine became transient in Ca-free (15 mM Mg) solution, and the norepinephrine effect declined exponentially with a half-time of 46 min by prolonging Ca depletion (1). ^{45}Ca efflux, probably reflecting intracellular Ca release, in response to norepinephrine decreased also exponentially, but with a half-time of 21 min. Thus, it was concluded that norepinephrine increases Ca influx and intracellular Ca release and that this results in an increase in K permeability.

Similar results were obtained with the *rat* aorta (382). In this tissue, norepinephrine increases Na, K, and Cl effluxes, as observed in the *rabbit* ear artery (121). In the aorta, Ca removal in the presence of 10 mM Mg inhibited the increase in ^{42}K and ^{36}Cl effluxes caused by norepinephrine, without much effect on ^{24}Na efflux. When Ca was removed or diltiazem was applied, the inhibition of contraction was closely correlated with that of ^{42}K efflux, both for the early phasic contraction and the late tonic contraction. This result suggests that the increase in K (and probably Cl) efflux is caused by intracellular Ca supplied from the external medium as well as from intracellular stores (382).

In the *rabbit* ear artery, norepinephrine (10 μM) increased ^{86}Rb efflux in two phases, an initial large increase and a small sustained increase (67). When norepinephrine was applied during exposure to excess K, ^{86}Rb efflux was inhibited after a transient increase. In normal solu-

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tion, the initial component was decreased when the external Ca concentration was reduced, while the late component was largely independent of Ca. However, following a prolonged (about 60 min) exposure to Ca-free solution, norepinephrine failed to affect the ^{86}Rb efflux. These results also suggest the regulation of K permeability by intracellular Ca.

Several mechanisms are probably involved in the action of catecholamines on K efflux. The Ca-activated K channel is likely to be one of the pathways (or the main pathway) for the increase in K efflux at least in the aorta and in the taenia, and this should be confirmed with electrophysiological methods in other types of smooth muscles. Before we can apply the results from radioisotope experiments to the analysis of membrane properties, it seems necessary to gain a better understanding of the kinetics of ionic fluxes measured with this method in multicellular tissues, like smooth muscle.

4. Veins.

The *canine* veins could be divided into two groups according to their sensitivity to norepinephrine, 5-HT, and histamine. The veins which originate embryologically from the body wall (external jugular, brachiocephalic, azygos, femoral veins, and superior vena cava) are sensitive to norepinephrine and 5-HT, whereas those originating from the intestinal system (portal, inferior vena cava just beneath the diaphragm, mesenteric, and pulmonal veins) are highly sensitive to histamine, but less sensitive to norepinephrine (184). In the *dog*, all veins obtained from 15 different regions seem to contain α_1-receptors judging from the response to phenylephrine, but the distribution of α_2-receptors is probably limited to some regions, because only 7 veins (saphenous, cephalic, jugular, mesenteric, femoral veins, inferior vena cava just below the liver, and portal vein) responded significantly to clonidine (372). The relative sensitivity to the agonists varied in these preparations; the sensitivity to phenylephrine was higher than that to clonidine in the portal vein and inferior vena cava, while the reverse was true in the saphenous, cephalic, femoral, and external jugular veins, probably related to their embryological origin. In longitudinal strips of the mesenteric vein, the sensitivity to the two agonists was similar.

Saphenous vein. In the *dog* saphenous vein, the effect of phenylephrine and norepinephrine and the high pA_2 value for prazosin (8.15 against phenylephrine, 8.48 against norepinephrine) suggest that both agonists activate postjunctional α_1-receptors (399). Furthermore, the agonists specific for α_1-receptors (SK & F 89748, *l*-1,2,3,4-tetrahydro-8-methoxy-5-(methylthio)-2-naphthalenamine, methoxamine) produced a much greater contraction than the specific agonist for α_2-receptors (BHT 920, 6-allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo(4,5-*d*)azepin dihydrochloride) (132). However, this tissue has atypical properties in that the blocking potency of yohimbine (pA_2 value, 7.51 against phenylephrine, 7.53 against norepinephrine) is about 10 times greater than is generally found for α_1-receptors. This peculiarity has been shown to be due to the coexistence of α_1- and α_2-receptors (89, 102, 132, 369). The presence of both α_1- and α_2-receptors was also demonstrated using agonists selective for α_1-receptors (cirazoline, St 587) and for α_2-receptors (xylazine, BHT 920, M-7, 2-N,N-dimethylamino-5,6-dihydroxyl-1,2,3,4-tetrahydronaphthalene, guanfacine, UK 14304, 2-(8-bromoquinoxalyl-7-imino)imidazoline tartrate), and antagonists selective for α_1-receptors (prazosin, phenoxybenzamine) and for α_2-receptors (rauwolscine) (129). The potency ratio of clonidine and phenylephrine and the order of potency of rauwolscine, yohimbine, and prazosin indicate that the *human* saphenous vein contains mainly α_2-receptors (391). However, a low slope of the Schild plot for rauwolscine and some inhibitory effect of prazosin on the contraction induced by norepinephrine suggest the existence of a small population of α_1-receptors. The contraction evoked by nerve stimulation was also more strongly inhibited by yohimbine than prazosin, suggesting that the neurotransmitter acts predominantly on α_2-receptors (114).

In the *dog* saphenous vein, both α_1- and α_2-agonists (phenylephrine and clonidine, respectively) produced fundamentally similar responses, i.e., an increase in ^{45}Ca uptake, and in Ca-free solution, a transient contraction accompanied by ^{45}Ca efflux (198). These results suggest that activation of α_1- and α_2-receptors causes both influx of Ca and release of intracellular Ca. Although verapamil (100 μM) shifted the dose-contraction curve of clonidine to the right more effectively than that of phenylephrine, this was interpreted that a high concentration of verapamil acts on the receptor level. However, there are many other reports which indicate some difference between the responses to α_1- and α_2-receptor activation in this vein. The contractile response to an α_2-selective agonist, M-7, was totally, while that to phenylephrine was only partially, abolished by Ca removal (238). Thus, the response mediated by α_2-receptors appeared to be more dependent on Ca influx. The results obtained with a Ca channel blocker, diltiazem, also support the importance of Ca influx for α_2-receptor activation (77). In these experiments, diltiazem (1 to 100 μM) preferentially inhibited the contraction caused by M-7 (α_2-agonist) and had little effect on that caused by cirazoline (α_1-agonist). The blocking action of nifedipine on the response mediated by α_2- (not by α_1-) receptors was also demonstrated (304).

Studies on the mechanical response and ^{45}Ca uptake caused by α_1- and α_2-selective agonists suggest that activation of α_1-receptors facilitates Ca influx and also causes intracellular Ca release, and that activation of α_2-receptors only causes Ca influx (202, 278). In these experiments, the response to 10 μM BHT 920 (a highly selective α_2-agonist) was accompanied by an increase in ^{45}Ca uptake. The contracture was nearly abolished (to about 3% of the control) by removal of the external Ca,

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whereas 100 μM phenylephrine still produced some response (14%).

The blocking action of nifedipine on the α_1-receptor-mediated response was studied more in detail by Jim et al. (201). The contractions evoked by various α_1-agonists were investigated in the presence of rauwolscine (α_2-blocker) and propranolol (β-blocker). Norepinephrine, phenylephrine, and SK & F 89748, acting as full agonists, produced similar maximum contractions, whereas the other agonists produced smaller maximum contractions (methoxamine > SK & F 87696 (5,8-dimethoxy-2-aminotetralin) = SK & F 88444 (5,8-dimethoxy-N,N-dimethyl-2-aminotetralin) > SK & F 88254 (8-methoxy-2-aminotetralin)) due to differences in intrinsic activity. The contractions evoked by these agonists were all affected significantly more with La (5 mM) than with nifedipine (1 μM). Since 5 mM La blocked ^{45}Ca uptake, the contractions evoked by norepinephrine and phenylephrine in the presence of La (about 50% of the control) were thought to be due to Ca released from intracellular stores. On the other hand, the contraction induced by SK & F 89748 was markedly decreased by La (to 17%), suggesting a large contribution by Ca influx. However, the contraction caused by SK & F 89748 was as resistant to nifedipine as the response to other full agonists. Nifedipine had a stronger depressing action on the contractions and ^{45}Ca uptake produced by partial agonists; i.e., the antagonism was stronger against an agonist with weaker intrinsic activity. The same conclusion has been reached using nimodipine as a Ca channel blocker, cirazoline as a full α_1-agonist, and St 587 as a partial α_1-agonist (90).

There are some complications in the relationship between changes in membrane potential and Ca influx (278). When the membrane potential was measured from the endothelial surface of the vessel with microelectrodes, activation of α_1-receptors by methoxamine or norepinephrine (prazosin sensitive) was more closely related to membrane depolarization, but caused intracellular Ca release in addition to Ca influx, whereas α_2-receptor activation by BHT 920 did not produce much depolarization, but was dependent on Ca influx which was suppressed by verapamil (1 μM) and nifedipine (1 μM). Changes in membrane potential in relation to activation of different receptor subtypes were further investigated (279). Norepinephrine (0.1 μM) produced 50% of the maximum contraction without causing depolarization, but higher concentrations depolarized the membrane. Selective agonists for α_1-receptors, methoxamine and SK & F 89748, produced depolarization dose dependently accompanied by contraction, and the ED_{50} values were nearly identical for depolarization and contraction. On the other hand, selective activation of α_2-receptors by BHT 920 and M-7 (up to 1 μM) had no significant effect on the membrane potential, although the contraction reached 80% of its maximum response. Thus, it was concluded that electromechanical coupling was mediated through α_1-receptors and pharmacomechanical coupling through α_2-receptors. Since the depolarization by BHT 920 was increased by TEA (10 mM), the small depolarization caused by α_2-receptor activation was considered to be due to an antagonizing action of Ca-activated K conductance resulting from an increase in Ca influx, as proposed for the *rabbit* pulmonary artery by Haeusler (153).

The *rat* saphenous vein produced ejps and a slow depolarization in response to nerve stimulation (81). The ejps were not blocked by prazosin or yohimbine. On the other hand, both the slow depolarization evoked by nerve stimulation and the depolarization caused by norepinephrine, BHT 920, or clonidine were antagonized by yohimbine, but not by prazosin. Phenylephrine and methoxamine produced neither a clear electrical nor mechanical response. These results suggest that α_2-receptors are responsible for the slow depolarization. Since the *dog* saphenous vein was more depolarized by activation of α_1-receptors than of α_2-receptors (279), there seems to be a species difference as to the proportion in which different receptor types contribute to the depolarization in the saphenous vein.

Mesenteric vein. In *guinea pig* mesenteric vein, norepinephrine (more than 1 μM) depolarized the membrane and generated electrical slow waves or action potentials, and the response was completely blocked by 5 μM phentolamine (401). The depolarization was associated with an increase in membrane resistance, probably due to a decrease in K conductance. However, since reduction of external Na depolarized the membrane from −64.2 mV to −50 mV and blocked the depolarization by norepinephrine, a contribution by a change in Na conductance to the depolarization is also likely. Reduction of Cl by substitution with glutamate did not modify the membrane potential nor the norepinephrine-induced depolarization. In this preparation, the only response to nerve stimulation was a slow depolarization, without a preceding ejp, and this was readily blocked by phentolamine (401). On the other hand, in the *dog* mesenteric vein, a single stimulus of perivascular nerves produced an ejp (403). When repetitive stimulation was applied, the ejp was facilitated and slow depolarization appeared at a frequency higher than 0.2 Hz. Yohimbine (0.1 μM) preferentially blocked the slow depolarization (403).

In *dog* mesenteric vein, norepinephrine (1 μM) depolarized the membrane and caused contraction (222). Yohimbine (higher than 0.1 μM) blocked these responses, but prazosin (up to 10 μM) partially inhibited the contraction only. Perivascular nerve stimulation produced ejp and slow depolarization. The ejp was partially reduced by 10 μM yohimbine, while the slow depolarization was completely blocked by 0.1 μM yohimbine. These results suggest that α_2-receptors are responsible for the slow depolarization in both *dog* and *guinea pig* mesenteric veins, and that the ejp is either mediated by the so-called

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"γ receptors" or evoked by a nonadrenergic cotransmitter.

Renal vein. In the *guinea pig* renal vein, norepinephrine (0.5 μM) depolarized the membrane by about 8 mV and decreased the membrane resistance by about 30%. These effects were blocked by yohimbine (0.1 μM), but not by prazosin (1 μM), suggesting involvement of α_2-receptors (266).

5. Summary.

The α-receptors activated by exogenous catecholamines are mostly of the α_1-type. Additional α_2-receptors are present in large arteries, but less prominent or absent in smaller arteries. In the *rat*, the vascular adrenoceptors seem to be atypical in that they possess α_1- as well as α_2-properties. The distribution of the two subtypes is not clear cut. It is not related to particular blood vessels, but may vary even in the same blood vessel among different species. The lack of specificity of both agonists and antagonists is probably one of the main factors preventing exact definition.

In electrophysiological experiments, agonists and antagonists have generally been used in high concentrations and a very limited range. Therefore, when they produce an effect, the conclusion is not necessarily reliable because of their nonselective action. There is, however, general agreement that the excitatory junction potential (ejp) evoked by sympathetic nerve stimulation is resistant to α-antagonists. The controversy of whether this is due to the presence of another adrenoceptor type (γ) or to the corelease of a nonadrenergic transmitter, such as ATP, has not yet been resolved. However, there is growing evidence for the involvement of a nonadrenergic cotransmitter.

The contraction and the slow depolarization of the membrane evoked by nerve stimulation are mediated not only by α_1- but also by α_2-receptors. Application of catecholamines can produce contraction without change in membrane potential or associated with depolarization. As described later, phosphoinositides in the plasma membrane may be involved in the contraction which is independent of membrane depolarization (i.e., the pharmacomechanical coupling), mediated through α_1-receptors (see section IX). Membrane events are not necessarily linked with contraction and may be modified via different receptor subtypes.

The contraction mediated by α_2-receptors is more strongly suppressed by Ca channel blockers than that mediated by α_1-receptors, where receptor-operated, voltage-independent Ca channels are involved (201a, 430, 431). In general, the contractions mediated through α_2-receptors depend strongly on Ca influx, while those mediated through α_1-receptors rely on intracellular Ca release in addition to Ca influx. This implies that α_1-receptors would be more effective in causing the phasic component of the contraction, while the tonic component would be evoked through α_2-receptors. This is largely what has been observed, but exceptions to this generalization are found when α_1-receptors are activated with partial agonists in some vascular muscles. This has also been demonstrated for the pressor response in the pithed rat and has been interpreted to be due to the presence of spare receptors (201a). However, in isolated preparations (*rabbit* aorta, *dog* saphenous vein), the idea of "spare receptor" does not seem to hold (45a). It is possible that the properties of Ca channels (e.g., their susceptibility to Ca channel blockers) are modified during agonist-receptor interaction, for example, by conformational changes of the proteins surrounding the channel. The changes produced by this interaction may depend not only on the receptor type but also on the agonists used. Thus, it may be that full agonists for α_1-receptors reduce the affinity of Ca channel blockers after agonist-receptor interaction.

B. β-Action

As in other smooth muscles, activation of β-receptors generally produces inhibitory effects on vascular muscles. One exception, recently reported, is that isoproterenol and norepinephrine both produced a very slow depolarization of the membrane (and presumably contraction) in the basilar artery of neonatal *rat* (60). No excitatory β-receptors were found in the adult *rat*.

1. Arteries.

The occurrence of β-receptor subtypes varies in different arteries and different species. For example, the *guinea pig* pulmonary artery contains only β_2-receptors, the *rat* and *rabbit* pulmonary artery and *rat* aorta contain predominantly β_2-receptors but also some β_1-receptors, and the *dog* left circumflex coronary artery contains β_1-receptors only (322).

Mesenteric artery. In the *guinea pig* mesenteric artery, isoproterenol (below 1 μM) did not modify the membrane potential, and only a small hyperpolarization (about 3 mV) was observed with more than 10 μM, although the threshold concentration reducing the resting muscle tone was 0.1 μM (192).

In the same preparation, the amount of Ca releasable from cellular stores was assessed by producing contractions with caffeine in Ca-free solution (192). The preparations were first exposed to Ca-free solution for 5 min, 2.5 mM Ca was then readmitted for various durations to refill the store, and thereafter 5 mM caffeine was applied for 2 min after Ca had been removed again. When isoproterenol (3 μM) was included in the Ca-loading solution, the subsequent caffeine contraction was increased. Thus, in this artery, isoproterenol seemed to increase intracellular Ca sequestration.

Ear artery. The effect of isoproterenol on Ca uptake was studied on the *rabbit* ear artery (429). A transient contraction produced by histamine (10 μM) in Ca-free solution was taken as an indicator of intracellular Ca release. When isoproterenol was added to the Ca-loading medium (40 mM K, 1.5 mM Ca), the subsequent histamine response observed 5 min after exposure to Ca-free

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solution was larger than the control without isoproterenol pretreatment, suggesting stimulation of Ca uptake into stores by isoproterenol.

Tail artery. The isoproterenol relaxation of the *rat* tail artery, contracted by 1 mM Ba, was suppressed dose dependently by ouabain. Removal of external K also inhibited the isoproterenol relaxation by approximately 80%. Furthermore, isoproterenol increased the degree of relaxation caused by readmission of 6 mM K during the norepinephrine contraction in 1 mM K, and ouabain (0.1 mM) blocked the K-induced relaxation in both the presence and absence of isoproterenol (443). Ouabain had no effect on the isoproterenol relaxation of *rat* tail artery contracted with KCl, although ouabain inhibited the relaxing effect of isoproterenol on *pig* tail artery contracted with KCl. While it is possible that stimulation of the Na pump is to some extent involved in the relaxing effects of isoproterenol in some mammalian smooth muscles, as suggested for the amphibian (*Bufo marinus*) stomach muscle (363), more direct evidence is necessary to establish this mechanism for the β-action. Furthermore, the contribution of the Na pump may vary greatly in different smooth muscles and in different species, and it very likely depends on the experimental conditions prevailing.

Coronary artery. In the *dog*, the large coronary artery has a high percentage of α-receptors compared with β-receptors, while the small coronary artery has almost exclusively β-receptors (86, 298). The β-receptors in *dog* and *pig* coronary arteries are of the β_1-type, as opposed to most other vascular muscles, in which the β_2-type is usually found (6, 24, 120, 203).

The smooth muscle cells of the *dog* coronary artery (1.5 to 2.0 mm in diameter) were hyperpolarized (6 to 10 mV), with a reduction of membrane resistance, by 1 μM norepinephrine or isoproterenol through activation of β-receptors (189). On the other hand, in the *dog* coronary artery (0.5 to 1.0 mm in diameter), isoproterenol (or phenylephrine in the presence of phentolamine) (up to 50 μM) reduced the resting tension without altering the membrane potential or the membrane resistance (190).

In Ca-free solution, the contraction of *rabbit* coronary artery, produced by 10 μM histamine, was increased by pretreatment with isoproterenol when it was applied during a preceding Ca-loading procedure in 30 mM K solution, probably due to a larger amount of Ca being available for release. When Ca loading was carried out in normal K (5.9 mM) instead of excess K, the pretreatment with isoproterenol reduced the subsequent histamine response in Ca-free solution. These results were interpreted to indicate that, under normal conditions, the main action of isoproterenol was inhibition of Ca influx, but that in excess K, it stimulated Ca uptake into the intracellular store (429).

In the *pig* coronary artery, isoproterenol inhibited the K contracture, but phosphorylase *a* activity remained at an elevated level during the relaxation (334, 335). Since the activity of this enzyme is Ca dependent, the intracellular free Ca concentration was considered to be sufficiently high for actin-myosin interaction. Thus, it was concluded that isoproterenol produced relaxation by reducing the sensitivity of the contractile machinery to Ca, which agrees with the conclusion reached in aequorin experiments on the *ferret* portal vein (300).

Aorta. In the *rabbit* aorta, depolarized by excess (80 or 145 mM) K (0 mM Na), isoproterenol (1 μM) produced relaxation accompanied by suppression of ^{45}Ca influx and an increase in cyclic AMP levels (290). These effects were mimicked by dibutyryl cyclic AMP (1 mM). No evidence was found for stimulation of Ca efflux by isoproterenol.

Carotid artery. In the *cat* carotid artery, K readmission following exposure to K-free solution produced relaxation which was blocked by ouabain, suggesting that activation of the Na pump was responsible for this relaxation. Since isoproterenol caused relaxation in K-free solution, which was not affected by ouabain, the Na pump did not seem to be involved in the relaxation caused by isoproterenol (45).

2. Veins.

Saphenous vein. Contractions of the *dog* saphenous vein evoked by acetylcholine (27 μM) were easily inhibited by isoproterenol, while those evoked by norepinephrine were more resistant. Similarly, acetylcholine contractions were much more inhibited by Ca removal or by verapamil than norepinephrine contractions. These results were interpreted as indicating that inhibition of Ca influx was the main effect of isoproterenol (87). Since the norepinephrine response is partly due to intracellular Ca mobilization, the isoproterenol effect is expected to be weaker. Although the K (80 mM) contracture was highly dependent on external Ca, it was relatively resistant to the relaxing effect of isoproterenol. This could be explained by the possibility that there are two different Ca channels. One type is activated by acetylcholine, less dependent on membrane potential, and sensitive to isoproterenol but less sensitive to verapamil. The other is activated by K, causing depolarization, and relatively insensitive to isoproterenol but highly sensitive to verapamil (87).

Facial vein. A large hyperpolarization was observed in the *rabbit* facial vein in response to isoproterenol, norepinephrine, as well as intramural nerve stimulation through activation of β-receptors (340). The membrane potential was increased from −46.8 to −70.8 mV with 10 nM isoproterenol, the relaxation being preceded by the hyperpolarization by about 200 ms.

3. Summary.

Effects on the plasma membrane. Activation of β-receptors hyperpolarizes significantly some vascular muscles (*pig* coronary artery, *rabbit* facial vein), but not others (*dog* coronary artery, *guinea pig* mesenteric artery). The

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significance of these differences between various vascular muscles and between different species remains to be clarified. Depending on the experimental method, observations might be partly determined by differences in density or homogeneity of the β-receptor distribution, which has already been discussed for the β-action on highly excitable visceral muscles. The importance of changes in the membrane function for the β-action, including hyperpolarization, is still not clear, but suppression of Ca influx seems to be the main factor involved in the β-action on *rabbit* aorta and *dog* saphenous vein. Activation of the Na pump has been suggested, because the relaxation by isoproterenol was blocked by ouabain or K removal. However, this could be a secondary effect, resulting from a significant accumulation of intracellular Na and Ca in unphysiological experimental conditions.

Intracellular effects. It is very likely that the β-action involves intracellular mechanisms in addition to an alteration of membrane function. The main action is probably the decrease of intracellular free Ca by an increased Ca uptake into stores, e.g., the SR. However, some doubts about this mechanism have also been raised; i.e., phosphorylase *a* activity, which is highly sensitive to Ca, was not affected during isoproterenol-induced relaxation in the *pig* coronary artery (334, 335). Therefore, if the properties of the contractile proteins were modified, for example, by cyclic AMP-dependent protein kinase, as described later, relaxation might take place without much decrease in the intracellular free Ca concentration (see section VIII).

A correlation between the types of blood vessel and the mechanism of the β-action has not been found, as it has for the α-action, but this point warrants further investigation.

VIII. Cyclic AMP and Catecholamine Action

Activation of both β_1- and β_2-receptors is known to stimulate adenylate cyclase, while activation of α_2-receptors inhibits this enzyme (393). The relaxation caused by β-agonists is thought to involve an increase in cyclic AMP (cAMP) formation by stimulating adenylate cyclase, because (*a*) the relaxation is correlated with a concomitant increase in cAMP content, (*b*) cAMP (applied as the dibutyryl compound) mimicks the effect of β-agonists, and (*c*) inhibitors of phosphodiesterase also have a relaxing effect. Intracellular cAMP controls smooth muscle contractile function by activating a protein kinase (11, 160). It may regulate the intracellular Ca distribution (302, 315). In addition, cAMP may suppress directly the contractile machinery by reducing the affinity of myosin light chain kinase for Ca-calmodulin (3, 4, 274, 288, 350). The affinity is reduced when the kinase is phosphorylated by cAMP-dependent protein kinase. Although the β-receptor-mediated relaxation is not necessarily correlated with an increase in cAMP, the evidence for an essential role of cAMP in the β-action is strong.

Forskolin, a diterpene isolated from the roots of *Coleus forskohli*, is known to stimulate adenylate cyclase directly, resulting in an increase of intracellular cAMP levels (365–367), and to relax smooth muscles (303). This provides a good pharmacological tool to investigate the role of cAMP.

A. Gastrointestinal Muscle

When the mechanical activity of the *rabbit* colon was suppressed by isoproterenol, there was a good correlation between the relaxation and the increase in cAMP. Furthermore, the mechanical effects of the β-agonist were mimicked by cAMP (10, 15).

In the *guinea pig* taenia, isoproterenol increased intracellular cAMP levels through activation of β-receptors. However, at a concentration of isoproterenol which reduced spontaneous contractions to 50% (ED_{50} = 5.5 nM), there was not always a significant increase in cAMP and, 10 s after the isoproterenol application, the relaxation reached 57% of the maximum before a significant increase in cAMP was detected. Furthermore, the pA_2 value (7.92) for propranolol, measured against isoproterenol-induced relaxation, was different from that (8.57) measured against isoproterenol-induced accumulation of cAMP. Thus, the relaxation mediated through β-receptors seemed to be a separate phenomenon, independent of cAMP accumulation (179). On the other hand, the effects of isoproterenol and forskolin were similar: both caused relaxation accompanied by a small hyperpolarization and an increase in ^{45}Ca efflux (106). Therefore, a contribution by cAMP to the β-action remains an attractive idea.

As described in section IV, the modification of electrical properties of the plasma membrane seems to be mediated through β_2-receptors, and the intracellular mechanism through β_1-receptors, at least in the guinea pig taenia (27, 216). However, the relative contribution of β-receptor subtypes to cAMP production remains to be clarified in each smooth muscle.

The actions of dibutyryl cAMP (0.3 mM) and isoproterenol (0.1 μM) are similar on the electrical and on the mechanical activity of the *guinea pig* taenia. But, in contrast to the decrease of the isoproterenol effect, the action of dibutyryl cAMP remained the same when Ca was substituted with Sr. In glycerinated (chemically skinned) muscle, contracted in the presence of 0.5 μM Ca and 4 mM Mg-ATP, dibutyryl cAMP (0.6 mM) was ineffective in suppressing the contraction. Therefore, it was thought that cAMP was not directly acting on the contractile machinery, but that it was either reducing Ca influx across the plasma membrane or inhibiting Ca release from Ca stores (409). On the other hand, isoproterenol and dibutyryl cAMP increased the loss of ^{45}Ca from the muscle, suggesting a possible involvement of Ca extrusion in the mechanism of relaxation caused by activation of β-receptors (419). In this experiment, the curve relating Ca concentration to tension, obtained by

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adding Ca to excess K (158 mM KCl, 6 mM $NaHCO_3$), was shifted to the right by dibutyryl cAMP (0.2 mM), but ^{45}Ca uptake by the preparation was not significantly decreased. However, in other experiments, in which NaCl was completely replaced with KCl (137 mM KCl, 6 mM $NaHCO_3$), isoproterenol (0.4 μM) lost its relaxing action, and concomitantly the increase in cAMP levels caused by isoproterenol was abolished (183). This loss of the action of isoproterenol may be due to the excessive increase in intracellular Ca.

The spontaneous contractions of *guinea pig* taenia were inhibited by cAMP (10 to 100 μM) (370), but, when studied with the sucrose-gap method, electrical and mechanical activities were not affected by dibutyryl cAMP at concentrations of 1 to 500 μM (135) or only weakly diminished at a concentration of 1 mM (53). Similar results were obtained in the circular muscle of *rabbit* colon, in which application of cAMP (0.4 to 3.2 mM) had a relaxing effect, while dibutyryl cAMP (1 mM) applied for 3 min produced no relaxing effect in 145 mM K (6 mM Na) medium (13). The reason why dibutyryl cAMP was effective in some experiments but ineffective in others remains to be clarified.

^{45}Ca accumulation by a microsomal fraction prepared from the *rabbit* colonic muscle was potentiated 2- to 3-fold by isoproterenol (4 μM) or by cAMP (10 μM) in the presence of 0.35 mM ATP (15). The ATP concentration in the medium seems to be critical for these potentiating effects. When the ATP concentration was high (5 mM), cAMP had no effect on Ca accumulation. Furthermore, stimulation of adenylate cyclase by isoproterenol (2 μM) was observed only at 0.35 mM but not at 5 mM ATP (314). It would be important to characterize the microsome, such as the sideness of the membrane formed, to know the distribution of β-receptors and to correlate the results with the β-action in the intact preparation.

The microsomal fraction, derived from the sarcolemma and sarcoplasmic reticulum of *guinea pig* taenia, was phosphorylated, and its Ca uptake was increased when pretreated with cAMP and cAMP-dependent protein kinase (172). The sarcoplasmic reticulum, rather than the sarcolemma, is probably responsible for this Ca uptake, because the enhancement of Ca uptake by the microsomes was observed only when oxalate was added, and Ca uptake by the sarcolemmal vesicles was not stimulated by oxalate. These results suggest that the mechanism underlying the relaxation caused by isoproterenol is a decrease in the free myoplasmic Ca concentration by Ca extrusion or sequestration.

In the *guinea pig* taenia skinned with 1% Triton X-100, in which the sarcoplasmic reticulum is not functioning, tension development was inhibited by cAMP, and this action was mimicked by the catalytic subunit of cAMP-dependent protein kinase (350). cAMP (100 μM) produced a maximal relaxation of 75% (25°C) in the presence of 0.53 μM Ca and 0.05 μM calmodulin, but this effect decreased with increasing Ca concentration. It was therefore suggested that the relaxing effect of cAMP is first to reduce the myoplasmic free Ca concentration and then probably to act directly on the actin-myosin system as the Ca concentration is lowered (288).

B. Airway Smooth Muscle

Isoproterenol increased the cAMP content, and there was a close correlation between the changes in cAMP levels and the degree of relaxation in the carbachol-contracted *canine* bronchial 423), the *bovine* tracheal (12, 212), and *guinea pig* tracheal (324) muscles. In the *canine* bronchial muscle, contracted by carbachol, dibutyryl cAMP (1 to 2 μM) and isoproterenol (0.1 μM) had similar relaxing effects (423).

Using six different methylxanthine derivatives and one nonxanthine agent (SQ 20,009 (1-ethyl-4-(isopropylidenehydrazino)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid, ethyl ester)), a strong correlation was found between phosphodiesterase inhibition and the relaxation of *dog* tracheal muscle, contracted by 0.1 μM methacholine, when one of five different phosphodiesterases separated by column chromatography was selected (338). Essentially similar results have been obtained from *guinea pig* tracheal muscle (134). In *bovine* tracheal muscle, the relaxant effect of isoproterenol was weaker when the contraction was produced with carbachol than with histamine. Similarly, the increase in cAMP by isoproterenol was less in the carbachol-contracted preparation (12, 253).

There is, however, not always a good correlation between the degree of relaxation and the increase in the level of cAMP. For example, in *bovine* tracheal muscle, contracted by carbachol (0.1 to 0.3 μM), isoproterenol (4 μM) and theophylline (a phosphodiesterase inhibitor, 10 mM) did not significantly elevate cAMP levels even though they reduced the contraction by 85 to 90% (253). In *bovine* trachea, contracted by methacholine (0.15 μM), salbutamol (β_2-agonist, 0.28 μM) and carbuterol (0.2 μM) failed to increase cAMP, although they produced maximum relaxation (243). Furthermore, β_2-selective blockers (butoxamine and $H_{35/25}$) antagonized the increase in cAMP but not the relaxation produced by isoproterenol. With several β-agonists, such as isoproterenol, epinephrine, terbutaline (β_2-agonist), and soterenol, the time-response relationship for relaxation and for increased cAMP content was generally parallel. Nevertheless, sometimes a clear dissociation between the relaxation and the increase in cAMP occurred.

In the *guinea pig* tracheal muscle, the degree of relaxation caused by isoproterenol decreased when the preparations were exposed to increasing concentrations of carbachol (51a, 211, 215a). The relaxing potency of theophylline, however, was less affected compared with that of isoproterenol when the carbachol concentration was increased (211), suggesting that their action is not simply mediated by a common mechanism resulting from an

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increase in intracellular cAMP. The antagonizing effect of carbachol on the increase in cAMP level caused by isoproterenol was much weaker than that on the isoproterenol-induced relaxation, and when the external Ca was removed, the carbachol effect disappeared (452a).

Studies of Ca uptake by microsomes (oxalate sensitive) and plasma membrane obtained from *bovine* tracheal smooth muscle failed to show a clear effect of cAMP and protein kinase (361). These experiments are difficult to evaluate, because of the poor characterization of the microsomes, whose properties may be influenced by many experimental factors.

When the catalytic subunit of cAMP-dependent protein kinase was added, purified myosin light chain kinase or endogenous myosin light chain kinase in a homogenate of the *bovine* tracheal muscle was phosphorylated, and as a result, the Ca sensitivity of the myosin light chain kinase was decreased. However, the Ca sensitivity of the kinase was unchanged when the endogenous cAMP-dependent kinase was activated by the addition of cAMP (5 μM). Furthermore, isoproterenol (0.3 μM), applied to intact muscles, either relaxed or contracted by carbachol or KCl, failed to affect the myosin light chain kinase activity. These results suggest that the relaxation by activation of the β-receptors does not necessarily involve phosphorylation of the myosin light chain kinase (295). On the other hand, in both relaxed and methacholine (1 μM)-contracted *canine* tracheal muscle, forskolin increased the cAMP level and also phosphorylated the myosin light chain kinase. This was taken to indicate that cAMP produced relaxation at least partly by this mechanism (101). Moreover, in *cat* tracheal muscle skinned with saponin, simultaneous application of cAMP (0.1 mM) and cAMP-dependent protein kinase (50 μg/ml) reduced contractions evoked by 0.3 to 10 μM Ca (188). These observations support the hypothesis that inhibition of the actin-myosin interaction may be the result of phosphorylation of myosin light chain kinase by protein kinase.

C. Myometrium

In pregnant *rat* myometrium, the cAMP content was more than doubled after 30-s exposure to 0.4 μM isoproterenol (227, 273), and the β-effects (relaxation and hyperpolarization of the membrane) were mimicked by dibutyryl cAMP (1 mM) (226), supporting previous observations on the estrogen-primed *rat* myometrium (422). It was therefore considered that cAMP played a major role in the β-action. The increase in cAMP levels in response to isoproterenol became markedly greater during the middle stage of gestation, particularly in the longitudinal muscle of *rat* myometrium. In the circular muscle, a significant increase of cAMP content by isoproterenol was also observed after the 21st day of gestation (219). These changes are in accord with the development of responses mediated by β-receptors (214, 219, 331).

Under some experimental conditions, a close correlation between the tissue content of cAMP and the degree of relaxation was not necessarily found. The cAMP levels of the estrogen-primed *rat* myometrium were increased (by 40%) during contracture produced in excess (125 mM) K (23.8 mM Na) solution, and this was considered to be due to release of endogenous norepinephrine, or due to stimulation of adenylate cyclase by an increase in intracellular Ca. Isoproterenol (5 nM) caused significant relaxation (by 35%), but increased cAMP only by an additional 16% over the depolarized condition (109). A clearer dissociation between cAMP levels and relaxation was seen when the estrogen-primed *rat* myometrium was exposed to 127 mM K solution containing no Na (Tris buffer) (434). Even at a very high concentration (100 μM), isoproterenol did not significantly increase cAMP, although the K contracture was clearly relaxed. The importance of Na in the increase of cAMP was confirmed by Meisheri et al. (289). The degree of relaxation produced by isoproterenol (100 μM) was similar in 47.5 mM K (80 mM Na), in 47.5 mM K (0 mM Na, sucrose substitution), and in 127 mM K (0 mM Na) media, but an increase of cAMP was 337% in the presence of 80 mM Na and only 100% in the absence of Na, and 600% in normal medium. On the other hand, isoproterenol increased cAMP, to a similar degree to the control, when the K contracture was prevented by D 600 (10 μM) or by removal of Ca (286, 289, 434). It is possible that Ca exerts an inhibitory effect on the adenylate cyclase complex and that activation of β-receptors removes Ca from this inhibitory site and activates the cyclase (286). One cause for a dissociation between cAMP levels and relaxation might be excessive accumulation of intracellular Ca.

Under normal conditions, isoproterenol inhibited the spontaneous mechanical activity of *rat* myometrium at much lower concentrations than that needed to increase cAMP. The increase in cAMP production was more closely correlated with binding of isoproterenol to β-receptors than with relaxation. Thus, occupancy of less than 10% of the receptors and a correspondingly small elevation of cAMP levels accounted for 50% of the maximal relaxation caused by isoproterenol (224).

In pregnant *rat* myometrium, forskolin was 10 times less effective than isoproterenol in suppressing spontaneous and K-induced contractions, but it increased cAMP significantly more than isoproterenol at equieffective doses. It was suggested that activation of β-receptors can inhibit the tension development via cAMP-dependent as well as cAMP-independent mechanisms (272).

In cultured myometrium cells from the *rat*, ^{45}Ca efflux was increased by isoproterenol dose dependently. This effect was blocked by propranolol and mimicked by 8-bromo-cAMP. Using broken cell membranes, it was found that adenylate cyclase was stimulated by GTP (300 μM) and by isoproterenol (ED_{50} = 0.5 μM) in the

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presence but not in the absence of GTP. Thus, guanyl nucleotides were required for the activation of adenylate cyclase through β-receptors (131). It was also found that adenylate cyclase was stimulated directly by Ca (ED_{50} = 0.1 μM) without GTP, suggesting the existence of another receptor-independent mechanism for cAMP production which may play a role in autoregulation of intracellular Ca by increasing Ca efflux.

cAMP (5 μM) increased the accumulation of Ca into a microsomal fraction (mainly sarcoplasmic reticulum) prepared from *rat* (ovariectomized) uterus by more than 50%. The increase in Ca accumulation by the microsomes was also demonstrated when the myometrium was treated with isoproterenol (100 μM) for 10 min prior to membrane isolation (225). Phosphorylation of a specific protein in the microsome is likely to be responsible for cAMP-dependent Ca accumulation in microsomes prepared from *rat* myometrium (315).

The spontaneous activity of estrogen-dominated *rabbit* myometrium was suppressed by 0.02 μM isoproterenol, unaccompanied by an increase in cAMP. At a higher concentration of isoproterenol (2 μM), cAMP was increased (259% increase after 3 min). In the presence of propranolol (3.4 μM) and phentolamine (0.53 μM), isoproterenol (2 μM) still inhibited the mechanical activity, but did not increase cAMP. The results were interpreted to indicate that isoproterenol stimulates β-receptors as well as some unknown receptors, both resulting in the suppression of mechanical activity, and that cAMP is not an obligatory mediator for relaxation (311). In a later study on the longitudinal muscle of *rabbit* myometrium, using the sucrose-gap method, it was found that dibutyryl cAMP (1 mM) and isoproterenol (1 μM) produced similar inhibitory responses. The possibility was considered that a small increase in intracellular cAMP, undetectable by the method used, is involved in the inhibitory action of isoproterenol (312). The hyperpolarization caused by dibutyryl cAMP was blocked by propranolol (3 μM). This observation needs further confirmation.

The estrogen-primed *rabbit* myometrium was much less sensitive than the pregnant *rat* myometrium to the cAMP-increasing action of isoproterenol and forskolin. Isoproterenol (0.005 to 0.5 μM) and forskolin (0.1 μM) reduced spontaneous and acetylcholine-induced contractions, without a significant change in cAMP levels, suggesting that cAMP was not an obligatory mediator (272).

In the pregnant *rat* myometrium and estrogen-primed *rabbit* myometrium, 1 mM dibutyryl cAMP mimicked the inhibitory action of isoproterenol on the mechanical and electrical responses, as already mentioned, while in the estrogen-treated *guinea pig* myometrium, 1 mM dibutyryl cAMP produced an excitatory action, similar to that mediated by α-receptors (53). It is likely that derivative compounds of cAMP applied exogenously may have a different action from cAMP which acts intracellularly through activation of β-receptors.

D. Vascular Smooth Muscle

Relaxation mediated by β-receptors in the *canine* coronary artery was accompanied by an increase in cAMP content. Dibutyryl cAMP (1 to 2 mM) and aminophylline (25 to 625 μM) produced relaxation in the muscle strip contracted by excess K (36 mM) (368). In the *bovine* coronary artery, relaxation with isoproterenol was highly correlated with an increase in both the cAMP levels and the activity of cAMP-dependent protein kinase, suggesting a causal relationship between these processes (376, 433). cAMP-dependent protein kinase phosphorylates specific proteins, such as contractile and Ca-transport proteins, and promotes relaxation. In the *porcine* coronary artery, treated with Triton X-100 for chemical skinning to destroy the function of the plasma membrane and the Ca-sequestrating system, contractions produced by Ca and calmodulin were antagonized by addition of the catalytic subunit of the cAMP-dependent protein kinase (0.01 to 0.5 μM) (336). The Ca required for half-maximal contraction was shifted from 1.1 μM to 6.3 μM by 0.5 μM protein kinase. These results support the idea that relaxation can be achieved by reducing the activity of myosin light chain kinase by cAMP, without a decrease in intracellualr free Ca concentration.

In the *bovine* coronary artery, forskolin (1 μM) produced the same effects as isoproterenol but, at a concentration of 0.1 μM, significant increases in cAMP and cAMP-dependent protein kinase activity occurred without relaxation. Furthermore, low concentrations of forskolin did not potentiate the effects of isoproterenol. These results were interpreted to indicate that either cAMP is not solely responsible for the relaxation, or there is a functional compartmentalization of cAMP and cAMP-dependent protein kinase in the coronary artery (432, 433).

In the *porcine* coronary artery, isoproterenol (5 μM) reduced the contraction produced by excess (118 mM) K without increasing the cAMP contents, although a clear increase in cAMP was observed in normal (5.9 mM K) medium. No enhancement of phosphorylation by isoproterenol was detected in the muscle homogenate, even under conditions in which cAMP levels were increased. Thus, the Ca regulation mediated through cAMP-dependent phosphorylation and the relaxation did not appear to be causally related (167). The basal activity of a particulate adenylate cyclase from *bovine* coronary arteries was strongly inhibited by 1 μM diisopropyl-fluorophosphate (DFP). The stimulating effect of isoproterenol (1 μM) on the cAMP synthesis was abolished by DFP (10 μM), even in intact tissues. On the other hand, the relaxation induced by isoproterenol was not affected by DFP. These results do not support the idea that β-adrenergic relaxation is mediated by cAMP (128). It would be well worthwhile to extend this kind of experiment to other smooth muscle types.

In the *rabbit* pulmonary artery, contracted by sero-

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tonin (62 μM), the relaxing effects of isoproterenol (8 μM) and theophylline (1.6 mM) were not correlated with an increase in cAMP (97). In this preparation, the activity of phosphodiesterase, rather than that of adenylate cyclase, seems to play an important role in controlling intracellular levels of cAMP.

In the *rat* aorta, forskolin (0.5 μM) decreased the contraction caused by 1 μM norepinephrine and increased the level of cAMP (251). The relaxation by forskolin was very slow, reaching a steady state after more than 15 min, and was much slower than the increase of the cAMP level and of the activity of the cAMP-dependent protein kinase. The difference in their time course needs to be clarified. The contractions caused by 0.1 μM norepinephrine and 117 mM KCl were similarly reduced by 0.5 μM forskolin (44.7 to 54.6%) and also by 100 μM dibutyryl cAMP (60.3 to 65.2%), but the mechanism underlying the inhibition is not clear. In other experiments, a clear difference was found between the effects of forskolin on the contractions induced by norepinephrine (3 μM) and KCl (80 mM) (205). The concentration of forskolin required to inhibit the norepinephrine contraction was significantly lower than that required to decrease the K contracture. When the norepinephrine contraction was reduced by 50%, the cellular cAMP levels were increased by 40%, and 90% inhibition was observed with a 2- to 3-fold increase in cAMP content. The tissue cAMP levels associated with the inhibition of the K contracture were 6 to 10 times higher than those observed with the inhibition of the norepinephrine contraction to the same extent. Thus, a direct action of cAMP on the contractile machinery is unlikely to be a major mechanism in relaxation. Since forskolin reduced ^{42}K and ^{36}Cl effluxes stimulated by norepinephrine at concentrations similar to those which were effective for mechanical inhibition, it is more likely that cAMP is reducing the intracellular Ca which controls the membrane permeability.

In the *rabbit* aorta, contracted by 145 mM KCl (0 mM Na), the relaxation caused by isoproterenol (1 μM) was accompanied by an increase in cAMP levels and inhibition of Ca influx, but Ca efflux was not affected. A phosphodiesterase inhibitor, Ro 20-1724 (1 mM), also caused relaxation, increased cAMP levels, and inhibited Ca influx. The effects of isoproterenol were potentiated by Ro 20-1724. Dibutyryl cAMP (1 mM) caused slow relaxation and also inhibited Ca influx. These results support the involvement of cAMP in the relaxation mediated through β-receptors, and the mechanism is supposed to be suppression of Ca influx (290).

The phosphorylation of a protein component of microsomes obtained from *rat* aortic smooth muscle was augmented by cAMP-dependent protein kinase. Ca uptake into microsomes was enhanced when they were phosphorylated in the presence of 1 μM cAMP or 1 μM cAMP plus 0.1 mg/ml protein kinase (35). This process is considered to be an important mechanism for the relaxation mediated by cAMP.

Interaction of cAMP at the site of the contractile protein through cAMP-dependent protein kinase is also proposed as a possible mechanism for β-receptor-mediated relaxation (34). Purified myosin light chain kinase, from the *bovine* carotid artery, was phosphorylated by cAMP-dependent protein kinase (20 μg/ml). This decreased the myosin light chain kinase activity, increasing 3- to 5-fold the amount of calmodulin required for half-maximal stimulation of the kinase. A similar conclusion, i.e., suggesting interaction at the contractile protein, was reached by using muscles of *porcine* carotid and coronary arteries, skinned by Triton X-100. In these preparations, it was demonstrated that cAMP (in the presence of 0.1 mM isobutylmethylxanthine) as well as the catalytic subunit (70 μg/ml) of cAMP-dependent protein kinase strongly reduced contractions produced by Ca (0.2 to 0.5 μM) in the presence of calmodulin (348, 349).

In the *rabbit* mesenteric artery, dibutyryl cAMP could produce two different effects (359). One effect was to inhibit the tonic phase of the norepinephrine contraction, probably the result of a reduction of the intracellular free Ca concentration through stimulation of Ca extrusion or Ca sequestration. Another effect was to increase the norepinephrine contraction probably by facilitating Ca-induced Ca release from the sarcoplasmic reticulum. Which of the two effects was observed was assumed to depend on the amount of Ca accumulated in the store. When the Ca content of the sarcoplasmic reticulum was more than a certain level, cAMP would predominantly stimulate the Ca-induced Ca release, thereby potentiating the contraction.

In the *guinea pig* mesenteric artery, Ca accumulation into store sites was increased by isoproterenol (0.1 μM), accompanied by an increase in cAMP levels. The Ca concentration-tension relationship in the fibers skinned with saponin was not affected by cAMP (3 μM) plus cAMP-dependent protein kinase (50 μg/ml), but the Ca-induced contraction was suppressed when the cAMP concentration was increased to more than 10 μM. The amount of Ca stored intracellularly was estimated from the size of the caffeine-induced contraction observed in Ca-free solution after the Ca-loading procedure. When the store was loaded with a low Ca concentration (1 μM) for less than 2 min, cAMP (3 μM) and protein kinase (50 μg/ml) potentiated the loading, but they had the opposite effect with higher Ca concentrations and longer loading periods, probably due to activation of Ca-induced Ca release (192). Similar results have been obtained with the *rabbit* mesenteric artery (193). Thus, in these muscles, the relaxation caused by isoproterenol appears to be mainly due to Ca sequestration mediated by a cAMP system, and the direct inhibitory effect on the contractile protein seems to be minor.

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In the *rat* and *pig* tail arteries, dibutyryl cAMP (10 μM to 1 mM) potentiated the relaxation induced by K readmission following an exposure to K-free solution. Isoproterenol (1 nM to 10 μM) had the same effect, and this was interpreted by assuming that isoproterenol caused relaxation partly by activating the electrogenic Na pump and that this was mediated by intracellular cAMP (443). It has also been shown that the Na-K-ATPase activity of microsomes prepared from *canine* mesenteric arteries was activated by isoproterenol (1 μM) and also by cAMP (1 nM to 1 μM) (250). Although some contribution of the Na pump to the β-action cannot be ruled out, this is probably not playing a major role (see also section VII).

In the *dog* saphenous vein contracted by methoxamine (10 μM), isoproterenol (3 μM) produced a marked relaxation (93%) and increased the cAMP content from about 0.2 to 0.45 nmol/g wet weight. On the other hand, in the *dog* portal vein, only a weak relaxation (about 25%) was observed by 3 μM isoproterenol, although cAMP was significantly increased (from 0.25 to 0.4 nmol/g wet weight) (215b). Essentially similar results were obtained with forskolin: methoxamine contraction was completely inhibited by 1 μM forskolin in the saphenous vein, whereas it was only reduced by 30% by 10 μM forskolin in the portal vein. The increase in cAMP was, however, over 4-fold higher in the portal vein than in the saphenous vein. Furthermore, dibutyryl cAMP (1 mM) had a marked inhibitory effect on the sephenous vein, but only a very weak effect on the portal vein. These differences were considered to be related to the fact that the portal vein belongs embryologically to the gastrointestinal muscle.

E. Summary

It is clear that adenylate cyclase is linked with β-receptors and that cAMP production is increased by β-receptor activation. The relaxation mediated by β-receptors is likely to be, at least partially, mediated through a cAMP-dependent process. But, there are some observations indicating no clear correlation between an increase in cAMP and the relaxation caused by β-receptor activation. This may be due to the fact that some cAMP-independent process is also involved. However, the lack of correlation can be explained by many other possibilities. (*a*) Adenylate cyclase may be located at several different sites, and only at one site is it functionally important for relaxation. Then, if an increase in cAMP content is sufficient at the critical site, this might not be detectable with the methods employed, if this compartment is relatively small. Such an idea could explain the discrepancy between the effects of forskolin and isoproterenol, especially if the degree of activation of adenylate cyclase by the two agents is not the same in different compartments. (*b*) An increase in cAMP content may not necessarily be the result of an increased rate of turnover which is much more important for the regulatory function of cAMP. Even when no clear increase in cAMP concentration is observed, the relaxation may nevertheless be achieved by its increased rate of turnover. (*c*) Many different coupling mechanisms between cAMP and relaxation may exist, as has been proposed for different preparations, and it is likely that the relative contribution of each mechanism varies in different types of smooth muscle and also depends on the experimental conditions. The regulatory efficiency of cAMP is probably different in each mechanism involved in relaxation.

cAMP is likely to reduce the free Ca concentration and modify the contractile protein, but in addition, it seems to act also at the plasma membrane, by hyperpolarizing the membrane and suppressing spike activity, judging from the similarity of the effects of isoproterenol and dibutyryl cAMP on some smooth muscles. However, the effects of cAMP on the cell membrane have not been analysed as much as its other intracellular effects. The contribution of cAMP to the β-action on the plasma membrane is one of the most interesting areas to investigate.

Another point worth investigating would be to find the relative contribution by different β-receptor subtypes to each mechanism involved in the processes of relaxation and of cAMP production. It is not known whether the predominant subtype contributes to both processes to the same degree.

IX. Involvement of Phosphoinositides in the α-Action

Phosphatidylinositol (PI), phosphatidylinositol-4-monophosphate (PIP), and phosphatidylinositol-4,5-bisphosphate (PIP_2) account in most cells for less than 10% of the total phospholipid contents. All three are found on the inner leaflet of the cell membrane, and their turnover is thought to have an important role in the sequence of events between the interaction of agonists with receptors and the final Ca-mediated response of the cell (31, 32, 294, 342). The agonist-receptor reaction activates phospholipase C, which catalyzes the hydrolysis of PIP_2 in the plasma membrane. The cleavage results in the formation of *myo*-inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DG). The phosphorylation of DG leads to the formation of phosphatidic acid. IP_3 may act as a second messenger to trigger the Ca release from intracellular stores, i.e., the sarcoplasmic reticulum (SR) (63). DG may mediate other intracellular processes by activating Ca- and phospholipid-dependent protein kinase C and by promoting arachidonic acid metabolism (316, 317). There is growing evidence for the hypothesis that PIP_2 hydrolysis is the initiating step in the mechanism by which α-receptor activation elicits the smooth muscle contraction (412). The protein kinase C activated by DG may increase the sensitivity of the contractile protein to Ca and is probably involved in the slow sustained phase of the norepinephrine contraction in vascular muscles (343). A phorbol ester, 12-O-tetradeca-

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noylphorbol-13-acetate (TPA), is known to be an activator of protein kinase C (316, 317). In the *porcine* coronary artery, TPA (0.1 μM) potentiated the K (39 mM) contracture. This effect was also observed on the tension development at low Ca concentrations (0.1 to 0.3 μM) in the saponin-skinned preparation. Since TPA had no effect on the intracellular Ca transient, measured with quin-2 (2-methyl-6-methoxy-8-nitroquinoline), during the K contracture, TPA may act on the regulatory mechanism of the contractile protein by activating protein kinase C (194).

A. Nonvascular Muscles

It has been shown that epinephrine (1 mM) decreased the tissue content of PI in the *rat* vas deferens (206), and that, through activation of α-receptors, the incorporation of ^{32}P into PI was stimulated in the *guinea pig* ileum by 125 μM epinephrine (197), and in the *rat* vas deferens by 0.1 to 1 mM norepinephrine (64), all suggesting an increase in the turnover of PI. Since very high concentrations of catecholamines were used in these experiments, it was important to demonstrate a quantitative relationship between the inositol response and lower catecholamine concentrations. In the *rat* vas deferens, the ^{32}P incorporation into phosphatidic acid was increased in a dose-dependent manner by norepinephrine (1 to 100 μM), and the amount of phosphatidic acid in the membrane was also increased by norepinephrine. These responses were significantly larger in denervated preparations which have a higher sensitivity to norepinephrine. It was concluded that phosphatidic acid may play a role in increasing Ca influx (413), as hypothesized by Michell (294).

Norepinephrine (0.1 to 100 μM) increased [^3H]inositol 1-phosphate (IP_1) accumulation in the *rat* vas deferens (ED_{50} approximately 3 μM) when incubated with [^3H] inositol for 3 h in the presence of 10 mM Li to block breakdown of *myo*-inositol-1-phosphatase, suggesting an increase in phosphatidylinositol turnover (133). The maximal contractions produced by partial agonists, such as clonidine and propanolamine, were about 50% of that caused by norepinephrine, but they were nearly as effective as norepinephrine in increasing [^3H]inositol 1-phosphate (IP_1). The reason for this discrepancy is at present not clear.

IP_3 released Ca from microsomes derived from the sarcoplasmic reticulum of pregnant *bovine* myometrium (65). At 5 μM, it released 40% as much Ca as a Ca ionophore, A23187 (calimycin, 0.2 μM) within 60 s.

B. Vascular Muscles

Norepinephrine, phenylephrine, and methoxamine increased [^3H]inositol phosphate production in the *rat* tail artery incubated with [^3H]inositol, through activation of α_1-receptors (133). Norepinephrine (1 to 100 μM) and methoxamine (10 to 100 μM) markedly increased the ^{32}P labelling of PI in the *cat* aorta as well (239). Similarly, α-stimulants increased the ^{32}P labelling of PI and phosphatidic acid in the *rabbit* aorta. Epinephrine and methoxamine produced their maximal effect at a concentration of 30 μM, but phenylephrine failed to elicit the maximal increase of PI labelling, although it acted as a full agonist for contraction. The ED_{50} values of these agonists for contraction (approximately 0.1 μM) were lower than those for incorporation of ^{32}P into PI (more than 0.5 μM), suggesting the presence of spare receptors. Prazosin was about three orders of magnitude more potent than yohimbine in antagonizing the ^{32}P incorporation and contraction, indicating that these effects were mediated by α_1-receptors (436). These results were confirmed and extended to show that loss of ^{32}P from PIP_2 was rapid, being significant already 30 s after norepinephrine (10 μM) application, and was correlated with an increase in Ca efflux, whereas ^{32}P incorporation into phosphatidic acid was slow, reaching a peak 2 min after norepinephrine application (63). The possibility was considered that IP_3 produced from PIP_2 causes intracellular Ca release, while phosphatidic acid increases Ca influx.

Similar results were obtained with the *rat* thoracic aorta. Provided that degradation of IP_1 was inhibited by lithium, norepinephrine increased IP_1 accumulation with an EC_{50} of 0.1 μM, and this effect was antagonized by prazosin, not by yohimbine (248). The formation of IP_1 was not affected by nifedipine (1 μM) which inhibited only the tonic phase of norepinephrine contraction (362). The phasic component of the contraction mediated by α_1-receptors was therefore thought to be due to mobilization of intracellular Ca caused by increased PI turnover.

In the *rabbit* pulmonary artery, skinned with saponin or digitonin, contraction could be evoked repeatedly by IP_3 (0.5 to 30 μM) (388). The contraction evoked by IP_3 was sustained, while that evoked by caffeine (20 mM) was transient, probably due to the secondary relaxing action of caffeine. Ca release by IP_3, detected with a Ca-sensitive electrode, was not influenced by metabolic inhibitors of oxidative phosphorylation (KCN and oligomycin), therefore ruling out mitochondrial sites for the source of the mobilized Ca.

In saponin-skinned single muscle fibers, taken from the *porcine* coronary artery, micromolar concentrations of IP_3 released Ca from intracellular nonmitochondrial stores (397, 398). Studies on the microsomes of sarcoplasmic reticulum prepared from the *porcine* aorta showed that Ca release by 5 μM IP_3 occurred within 1 min, and that this release was most effective when the store had accumulated Ca to about half the maximum capacity (398). Ca release was inhibited when the Ca concentration outside the microsomes was higher than 1.5 μM. The Ca release by IP_3 in the saponin-skinned fiber was increased by ATP and its unhydrolyzable analogue, 5-adenylyl-imidodiphosphate (AMPPNP), but not by ADP or AMP. Since Ca-induced Ca release from

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the sarcoplasmic reticulum is increased as the store accumulates more Ca, and since it is stimulated not only by ATP and AMPPNP but also by ADP and AMP, it was concluded that the IP_3-induced Ca release is different from the Ca-induced release.

Similar results have been obtained from cultured *rat* aorta smooth muscle cells, skinned with saponin (381). In these experiments, IP_3 transiently increased ^{45}Ca release from intracellular stores (maximally at 20 μM), and the presence of ATP (maximum effect at 3 mM) was necessary for Ca release. Since unhydrolyzable ATP analogues (β,γ-imido and β,γ-methylene derivatives) were as effective as ATP and the effect of lowering the temperature was weak, it was considered that IP_3 activates a Ca channel in the membrane of the intracellular Ca store, independent of ATP metabolism. Using the same preparation (saponin-skinned, primary cultured smooth muscle cells of *rat* aorta), it was demonstrated that 10 μM IP_3 released Ca from intracellular stores with a half-time of less than 10 s, and that 100 μM IP_3 produced a maximal release of 97% of the Mg-ATP-dependent ^{45}Ca uptake, and this was much larger than that attained by caffeine (25 mM) (454). Since IP_3 was very effective compared with caffeine at a low free Ca concentration (1 μM), it was suggested that Ca release by IP_3 was independent of cytoplasmic Ca, while that by caffeine requires the presence of some Ca outside the sarcoplasmic reticulum.

In the *rabbit* mesenteric artery, PIP_2 was reduced and phosphatidic acid was increased by norepinephrine higher than 1 nM, and the maximum effect was obtained with 1 μM norepinephrine (161). Norepinephrine (10 μM) increased the synthesis of IP_3 within 10 s, reaching a peak at 30 s when measured with incorporation of *myo*-2-[^{3}H]inositol. The Ca-releasing action of IP_3 on intracellular stores was studied in saponin-treated muscle cells of the *dog* mesenteric artery dispersed with collagenase (161). After loading the store with ^{45}Ca in the presence of 0.3 μM Ca and 2 mM ATP, 3 μM IP_3 reduced the amount of stored Ca within 1 min. The contraction due to Ca release by IP_3 (0.1 to 10 μM) was demonstrated in the *rabbit* mesenteric artery skinned with saponin (161).

In the *porcine* coronary artery, the activity of the sarcolemmal Ca-ATPase was inhibited by IP_3 (0.2 to 1 μM (339). This suggests that IP_3 is very effective in increasing the intracellular Ca concentration because of a dual mechanism: Ca release from the sarcoplasmic reticulum and inhibition of the Ca pump.

C. Summary

It is highly probable that IP_3 is responsible for the early phase of the contraction mediated by α_1-receptors by releasing Ca from the sarcoplasmic reticulum (SR). The IP_3-induced Ca release from the SR is different from the Ca-induced Ca release, but whether Ca-induced Ca release is involved in the action of catecholamines is not clear. It will be interesting to see whether Ca-induced Ca release is perhaps involved in the response mediated by α-receptors, particularly the α_2-subtype. And the possibility remains, even for the α_1-receptor-mediated contraction, that some other process, including Ca-induced Ca release, is also involved in addition to the IP_3-mediated intracellular Ca release.

The mechanism for the late sustained phase of the norepinephrine contraction is still not clear. It is possible that protein kinase C activated by DG increases the efficiency of the contractile machinery so that a large contraction can be maintained at a low Ca concentration. However, it is also likely that an increased Ca influx is responsible for maintaining the late contraction, because of its high sensitivity to the external Ca concentration. No electrophysiological study is as yet available in relation to Ca influx across the plasma membrane which is involved in the late sustained phase of the contraction. This needs to be done to characterize the mechanism of Ca influx and to clarify the mechanism of action of Ca-channel blockers. It is not certain at the moment whether some endogenous Ca ionophores, such as products of arachidonic acid or phosphatic acid, are also involved in the excitatory action on vascular muscle, as well as in the inhibitory action on intestinal smooth muscle mediated by α_1-receptors. A linkage of the α-receptor to an ionophore for the inward Ca transport, which is facilitated by epinephrine, has been proposed for the *guinea pig* taenia (417). This problem is left for the future. Since the PI response in the α-action is one of the most interesting fields for investigation, one would expect to have a much clearer and definite view on its mechanism within a few years.

X. General Conclusion

The mechanisms which control the contractile activity of smooth muscles in the body are complicated. The action of catecholamines is only one factor in the concerted action of many substances which cause contraction or relaxation, at various speeds and to various degrees, according to the requirements for their specific function. Under physiological conditions, smooth muscle activity is considered to be mainly regulated by the Ca movement across the plasma membrane. An increase or a decrease of Ca influx is probably the most sensitive way of causing contraction or relaxation, and the most important action of catecholamines under physiological conditions is thought to be on the membrane.

When the mechanical activity is regulated by spontaneous spike activity, as that of the longitudinal muscle layer of the intestine, the muscle tone is increased or decreased by increasing or decreasing the spike frequency. When a brisk contraction of a quiescent muscle, like the vas deferens, is required, action potentials of large amplitude are generated to evoke the contraction. When a weak and slow contraction (or relaxation) is appropriate, as in large blood vessels, Ca influx is con-

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trolled by slow depolarization (or hyperpolarization) or without much change in membrane potential.

The Ca channel controlled by agonist-receptor interaction (the receptor-operated channel) is insensitive to the membrane potential, while that responsible for the generation of an action potential (the voltage-operated channel) is sensitive to the membrane potential (39, 425). One would expect that a membrane with a high receptor density is electrically less excitable, as pointed out by Grundfest (150, 151). In skeletal muscle, the acetylcholine receptors are mainly restricted to the end plate region which is surrounded by the electrically excitable membrane. In smooth muscle, however, the receptor-operated as well as voltage-operated channels seem to be distributed over the whole surface area of the cells, but their density and homogeneity are probably quite different in different types of smooth muscle. In order to solve these problems, the study of radioligand binding to the receptors is important.

Another factor which determines the excitability is, of course, the nature of ionic channels, particularly for Ca and K. Since the inward current responsible for the action potential is believed to be carried by Ca in most smooth muscles, the density and kinetics of the Ca channels are important in determining excitability. In vascular smooth muscles, it seems that the electrical excitability is closely correlated with the density of sympathetic innervation (166). It may be that, in highly excitable vascular smooth muscles, the receptor molecules are packed in clusters, as at the motor endplate of skeletal muscle, and that the adjacent membrane contains a high density of voltage-dependent Ca channels. On the other hand, in poorly excitable vascular smooth muscles, the receptors may be distributed homogenously, limiting the development of voltage-dependent Ca channels, as Grundfest suggested. In addition to these geometrical factors, some trophic substance may be released from the nerve to regulate Ca and/or K channels, as assumed by Hill et al. (166).

It is possible that the outward K current, which opposes the depolarizing action of the inward current, is a more important factor controlling excitability than the inward current. There is good evidence that a relatively high K conductance stabilizes the membrane at a high resting potential and that the K channel blockers, such as TEA or Ba, increase the excitability or generate spontaneous action potentials in many quiescent smooth muscles (e.g., tracheal or vascular). Excitatory agents, including catecholamines, may reduce the K conductance of the plasma membrane, while inhibitory agents may have the opposite effect, and the K conductance is probably controlled by Ca acting at the internal surface of the plasma membrane, which has been termed "Ca-activated K conductance." Thus, translocation of Ca to and from the plasma membrane should be investigated in relation to catecholamine actions, and the difference between the responses mediated through different receptor subtypes should be clarified with regard to this particular point.

The contractions caused by Ca influx through the channel controlled by receptors, or by Ca influx through Ca ionophores in the plasma membrane, or by Ca released intracellularly, are not expected to be significantly affected by modification of the K conductance of the plasma membrane. When contractions are evoked in experimental conditions that are not physiological (e.g., in excess K medium or using skinned muscle fibers), then the membrane effect is reduced or abolished. To suppress this kind of contraction, one has to have a substance which acts at the receptor site or at intracellular sites regulating contraction. Any mechanism that is proposed on the basis of observations under such unphysiological conditions may be involved in addition to that which modifies membrane function, but the most important control mechanism exerted by catecholamines is probably set in the plasma membrane.

It is quite possible that adrenoceptors actually present in various smooth muscles cannot be classified simply as four different subtypes (α_1, α_2, β_1, and β_2) but that they are composed of a spectrum of receptors with different properties. Therefore, the mechanism underlying the response may differ to some degree among different smooth muscles, even though it is mediated through receptors belonging apparently to the same subtype. A new idea is that there may be another receptor subtype, the so-called γ-receptor, which would be located close to the site of transmitter release from the nerve terminal and would be responsible for the excitatory junction potential in vascular smooth muscle (168, 169). Another suggestion is that the ejp may be due to ATP, released as a cotransmitter, as observed in the vas deferens (384). It is clearly most important for a better understanding of the nervous control of smooth muscles to analyse the properties and the ionic mechanism of the junction potential further. In addition, the influence of other substances, simultaneously released with the sympathetic nervous transmitter, will have to be considered.

The relationship between the catecholamine receptor, or receptor type, and the intracellular coupling mechanisms has not yet been fully analysed. Phosphatidylinositol may be involved in the contractile response mediated through α_1-receptors, and cyclic AMP may be involved in the inhibitory response mediated through β_1- as well as β_2-receptors. The contribution of these second messengers in the coupling process between the receptor and the contractile protein has been postulated and is increasingly likely. But their regulatory roles at the cell membrane are not well understood and may well be of equal importance. In highly excitable smooth muscles, it is possible that intracellular cAMP, increased by β-receptor activation, effectively inhibits Ca influx caused by membrane activity, but inhibits less effectively other

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mechanisms which increase the intracellular Ca concentration and activate the contractile machinery.

Another factor in the coupling processes between the receptor and the final response is the variable sensitivity of the contractile apparatus to Ca. Experiments with a Ca-sensitive fluorescent substance, aequorin, suggest that the tension development is not simply related to the intracellular free Ca concentration, as usually supposed (100, 300, 301). In the α-receptor-mediated contraction, some intermediate process seems to increase the efficiency of Ca binding to the contractile protein, probably via activation of protein kinase C (98, 343). This is a fascinating field to be explored.

It must be kept in mind that intracellular processes can vary even if the same receptor initiates the response. (*a*) The properties of the receptor or the events leading to the mechanical response can be modified in many ways, such as ionic environment, the temperature, the metabolic state of cells, the age, or hormonal state of the animal, etc. (*b*) It may be that several different intracellular processes are activated simultaneously or consecutively by the receptor-agonist interaction, probably in relation to the location of the smooth muscle in different organs and, hence, to the function. As already mentioned, the innervation, the distribution, and properties of receptors and the character of the mechanical responses vary according to function. This variability is one of the characteristics of smooth muscle, and it is also a reason for the difficulty of smooth muscle research, in addition to the technical difficulties due to the complex structure of the tissue. However, in general, lack of precision in the experimental approach and insufficient consideration of the special, individual mechanisms that, in each muscle, lead to the mechanical response may be greater factors causing confusion than the complexity of smooth muscle itself.

We have now reached a stage when new techniques can be applied, such as the patch clamp method, to analyze the properties of single ionic channels, accurate methods for measuring the intracellular free Ca concentration, advanced biochemical and histological techniques, and recent gene technology. We can, therefore, expect that, within a few years, we will be able to identify much more precisely the mechanisms operating in receptor-agonist interaction at the membrane, in ionic permeability changes, and in the intracellular regulation of the contractile machinery, including the involvement of intracellular second messengers, such as substances related to phosphatidylinositol, and the role of cyclic AMP.

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