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Catecholamines then and now

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This paper is not a review. It is a highly subjective selection from the literature on the action of catecholamines and describes some of the early observations which contributed to the subsequent development of our present concept of the—apparently ubiquitous—interactions between nervous transmitters. The subject was chosen because the beginning was made in the Pharmacological Research Laboratories of the Pharmaceutical Society in Bloomsbury Square, 40 years ago (for references covering this period, see Bibliography: Reviews).

It all started with the discovery by Burn (1932) that an infusion of adrenaline into the bloodstream restored the efficacy of sympathetic nerve stimulation. This restoration was first seen by Burn during the study of vascular responses in a perfused preparation of the hindlegs of the dog. Depending on various experimental conditions, stimulation of the lumbar sympathetic chain caused either vasoconstriction or vasodilatation. Both were improved by the addition of adrenaline to the circulation. The effect was interpreted by Burn as being due to the uptake of adrenaline by nerve terminals, thereby replenishing exhausted transmitter stores (Burn, 1976).

My collaboration with Burn began in 1933, and we continued the study of vascular responses, especially vasodilator responses, to sympathetic nerve stimulation comparing several animal species (Bülbring & Burn, 1935).

At that time, the sympathetic nervous transmitter had not been identified. It was called 'sympathin' or an 'adrenaline-like' substance and, in our experiments, the responses to sympathetic nerve stimulation were compared with those to injected adrenaline. Numerous discrepancies were, of course, observed but were not fully understood. (Little was known beyond the fact that ergotoxine abolished motor effects and sometimes converted motor into inhibitory effects.)

Skeletal muscle

Since the sympathetic vasodilator innervation was mainly confined to the muscles and, in our experiments, the muscles were at rest, there was no clue as to its physiological function. Hence, an investigation was started of the blood supply during muscle activity and of the effect of sympathetic stimulation during muscle fatigue, the so-called 'Orbeli-phenomenon'. Orbeli had shown that, when frog muscle was stimulated through its motor roots until fatigue occurred, simultaneous stimulation of the lumbar sympathetic chain restored the force of contractions. In the dog it was found (Bülbring & Burn, 1939a) that, during muscle work, both sympathetic stimulation and adrenaline caused vasoconstriction, the vessels being already vastly dilated during muscle activity. However, a rise in muscle tension occurred, in spite of the diminution in blood flow. Moreover, since the relief of muscle fatigue by adrenaline was still seen after ergotoxine, it could not be due to the vascular effect.

The analysis was continued by experiments on non-fatigued muscle, applying not only tetani but also single shocks by direct and indirect stimulation. This led to the conclusion that the main effect of sympathetic stimulation and adrenaline was an improvement of neuromuscular transmission. There was a small augmentation of the force of contractions in directly stimulated muscle, thought to be on the muscle itself. A small effect was also exerted by other vasoconstrictor agents. This was thought, perhaps, to be an action on the nerve, and seemed to be connected with the restoration of a very low vascular tone.

A demonstration of the Orbeli phenomenon which was given to the Physiological Society at an Oxford meeting (Fig. 1) led to the first digression from classical pharmacological methods to electrophysiological methods. It was found, in experiments on cats, that adrenaline restored the excitability of the motor nerve by lowering the threshold (Bülbring & Whitteridge, 1941). When submaximal stimuli were applied, adrenaline increased the amplitude of the compound nerve action potential. This effect lagged behind and outlasted the vasoconstriction by adrenaline. It was seen in healthy nerve, but was much larger in fatigued nerve. '... a striking absence of parallelism between the effect of adrenaline on muscle and nerve was observed. When there was an increase in muscle

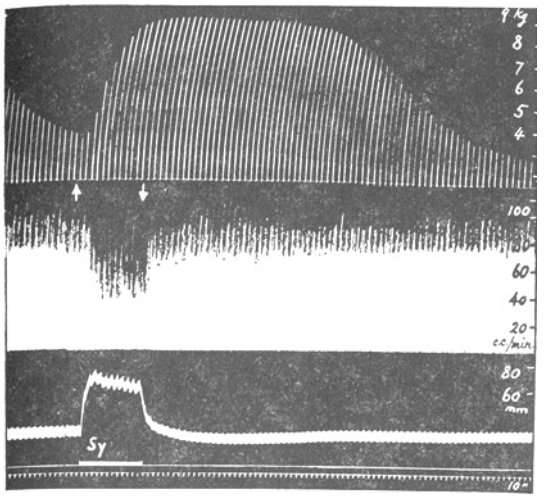

FIG. 1. Dog, perfused hindleg. The upper record shows the tension produced by the gastrocnemius muscle in response to single shocks applied to the motor roots. The middle record shows venous outflow. The lower record shows perfusion pressure. Between the arrows the lumbar sympathetic chain was stimulated for 2 min causing a prolonged increase in the muscle response (from Bülbring & Burn, *J. Physiol.*, 1939b with permission).

tension it always began earlier and outlasted the increase in the nerve response; sometimes a transient decrease of muscle tension was observed while the nerve action potential was growing. A further investigation of adrenaline on the nerve-muscle-junction is called for'!

The next step in this direction was the introduction of the isolated phrenic nerve-diaphragm preparation of the rat in which vascular effects were excluded (Bülbring, 1946). This showed that adrenaline increased the muscle response to nerve stimulation, (a) in maximally stimulated fatigued muscle, (b) in submaximally stimulated non-fatigued and fatigued muscle. The results indicated that adrenaline improved neuromuscular transmission. Furthermore, adrenaline augmented the potentiation of muscle contractions by prostigmine, confirming previous results obtained in perfused preparations and in the whole animal.

An electrophysiological investigation of the isolated phrenic nerve-diaphragm of the rat, using maximal stimulation only, showed that adrenaline increased twitch size and increased the duration of the muscle action potential, but not the amplitude (Brown, Bülbring & Delisle Burns, 1948). It was concluded that 'the action of adrenaline in the fatigued nerve-muscle preparation is primarily upon the muscle itself, and that effects upon neuromuscular transmission play only a small part, if any at all'!

So, by 1948, I found my name on a number of papers arriving at three different conclusions, i.e. that the action of adrenaline and of sympathetic nerve stimulation was due to an effect (a) on neuromuscular transmission, (b) on nervous excitability, (c) on the muscle itself.

Two major developments have led to our present concept of the action of catecholamines on skeletal muscle. The first was the introduction of intracellular electrical recording, the second was the introduction of specific pharmacological antagonists which allowed the separation of α- and β-effects of catecholamines.

In 1955 Hutter & Loewenstein repeated the original Orbeli experiment in the frog. They stimulated the motor fibres maximally and found that in fatigued or in partially curarized muscles sympathetic stimulation, or adrenaline, or noradrenaline increased both the muscle twitch and the muscle action potential. Most important, the intracellularly recorded endplate potential was increased. Since they also saw a potentiation of the endplate depolarization by acetylcholine, they concluded that the Orbeli effect was facilitation of neuromuscular transmission, but of postjunctional origin, i.e. a sensitization of the motor endplate to acetylcholine.

Three years later, in 1958, similar experiments were carried out by Krnjević & Miledi on the rat diaphragm. They complained in the introduction of one of their papers that 'the literature has been conspicuous for the variety of phenomena described and for the diversity of explanatory hypotheses'. Then they proceeded to add some more observations. They found, during fatigue, a presynaptic failure of conduction—i.e. some stimuli failed to elicit end-plate potentials—and a 100% restoration of transmission by adrenaline. Like Hutter & Loewenstein (1955), they saw an increased amplitude of the e.p.p. which they interpreted as more transmitter being released. In the mammalian preparation they found no postjunctional sensitization to acetylcholine but an acceleration of miniature e.p.p.s. On the other hand, the postjunctional threshold was first lowered, later it was raised, so that in their summary they state: 'This multiplicity and mutual interference between presynaptic and post-synaptic events may help to explain the variety of effects ascribed to adrenaline'.

Actually the first of a series of experiments which finally led to the explanation of these findings was

published in the same year, 1958, by Bowman & Zaimis. They were chiefly concerned with the differences between fast and slow muscles. In fast muscle, noradrenaline, adrenaline and isoprenaline, all increased twitch tension and prolonged twitch duration, in slow muscles they reduced the size and duration of the twitch. However, the slow (soleus) muscle was much more sensitive than the fast (tibialis) muscle, to adrenaline and isoprenaline, and these were 50–100 times more potent than noradrenaline—a clear indication that this was a β-action.

The final clarification, by separation of the α- and β-effects on the process of neuromuscular transmissions, is mainly due to the work of Bowman and his collaborators largely done in the School of Pharmacy laboratories. They showed that the action of catecholamines on skeletal muscle consists of an α-effect on the motor nerve ending and a β-effect on the muscle fibre. So, now it was possible to explain the great variety of phenomena which were observed in the course of 30 years (Bowman & Raper, 1966; Bowman & Nott, 1969).

The α-action on the motor nerve ending is probably the main factor in the improvement of neuromuscular transmission by adrenaline. It causes relief of presynaptic conduction failure and increases the release of acetylcholine. This is shown by the increased amplitude of the endplate potential (Fig. 2A, B) and by the increased frequency of minature e.p.p.s. (which explains the augmentation of repetitive firing in the presence of prostigmine).

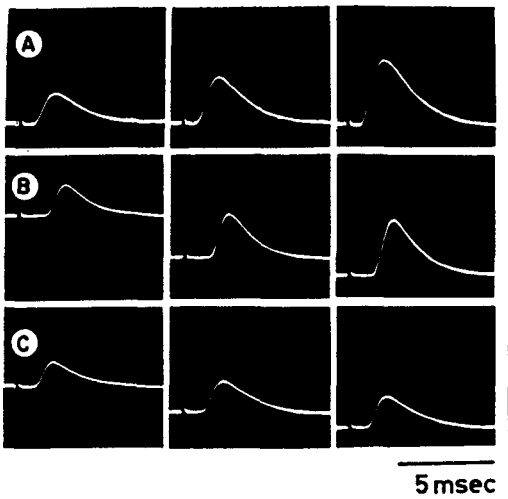

FIG. 2. Rat, phrenic nerve-diaphragm. Effect of catecholamines on end-plate potentials, in the presence of (+)-tubocurarine (1×10^{-6} g ml^{-1}). First record in each row, control, second 5 min, third 10 min after application of: A: noradrenaline (10^{-6} g ml^{-1}), B: adrenaline 10^{-6} g ml^{-1}, C: isoprenaline (10^{-6} g ml^{-1}). Note increase of e.p.p. caused by noradrenaline and adrenaline, and hyperpolarization of muscle membrane by adrenaline and isoprenaline. (Adapted from Kuba, *J. Physiol.*, 1970, with permission.)

The β-action on the muscle fibre itself can contribute to the improvement of muscle contractions. The active state of the fast muscles (the only ones investigated in our early work) is prolonged, i.e. the duration of the action potential is prolonged and, in parallel, both amplitude and duration of the twitch are increased. On the other hand, the muscle membrane is slightly hyperpolarized (Fig. 2B, C) and this may, in some conditions, raise the threshold of excitation sufficiently to block the spike, i.e. depress transmission because the e.p.p. does not reach firing threshold.

In recent years, more details have been filled in and identified as α- or β-actions, so that in the end, after many years of controversy, nobody has been wrong and everybody is right:—catecholamines influence skeletal muscle contractions by acting on both sides of the neuromuscular junction. The main sites of action are shown diagrammatically in Fig. 3.

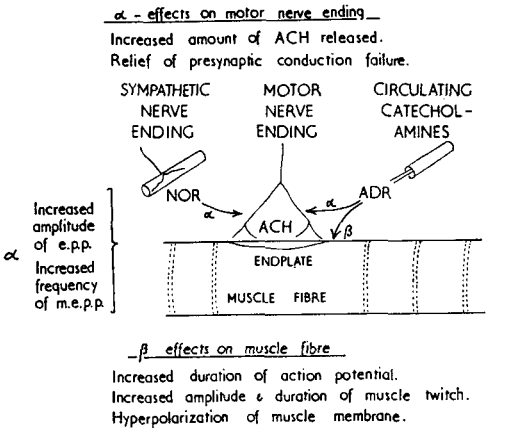

FIG. 3. Diagram of main actions of catecholamines on the neuromuscular junction in skeletal muscle.

The sympathetic ganglion

In the superior cervical ganglion the interaction of catecholamines and acetylcholine is built in by the existence of an adrenergic interneuron in the cholinergic preganglionic pathway. This pathway has recently been shown to be very complex (see Fig. 4). In addition to the classical nicotinic receptors on the principal ganglion cell, there are muscarinic receptors as well, not only on the ganglion, but also on the adrenergic interneuron which may release dopamine,

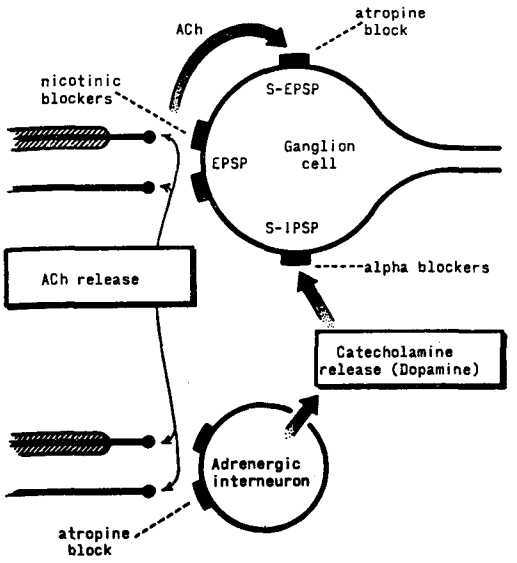

FIG. 4. Diagram of the interaction between acetylcholine and catecholamines in sympathetic ganglion (Reprinted from Libet, Fedn. Proc., 29, 6 1970).

causing hyperpolarization and thus inhibition of the ganglion. In this way the chromaffin interneurons (the small intensely fluorescent or SIF cells) probably exert a modulating influence on ganglionic transmission. The complex shape of the action potential is due to the super-position of the effects produced by the release of the different transmitters and consists of the following components:

(a) the initial excitatory potential, EPSP, due to the nicotinic action of acetylcholine;
(b) the slow excitatory potential, S-EPSP, due to the muscarinic action of acetylcholine;
(c) the slow inhibitory potential, S-IPSP, due to the action of the transmitter released by the SIF cells. This is abolished by α-blockers.

In early experiments, in which the superior cervical ganglion was perfused with Locke solution (Bülbring, 1944) it had been shown that adrenaline, in small doses, improved ganglionic transmission and in large doses caused depression. It seems now that the principal ganglion cell itself has α- as well as β-receptors. The α-action causes hyperpolarization. However, in normal conditions, this is small and it is not correlated with the degree of inhibition. The β-action, on the other hand, causes depolarization of the principal ganglion cell and facilitates ganglionic transmission—but only at muscarinic sites, not at the main nicotinic sites of the action of acetylcholine. In the perfused superior cervical ganglion, the improvement of ganglionic transmission by adrenaline may be explained if it is assumed that, in the exhausted ganglion, the facilitating β-action is dominant.

Adrenaline has an α-action on the preganglionic nerve terminals where it reduces the acetylcholine release and decreases the frequency of miniature potentials. Adrenaline has no effect on the threshold of presynaptic nerve endings, but is thought to act on the transmitter release mechanism involving calcium.

On the whole, the influence of catecholamines on the sympathetic ganglion appears to be the opposite of that on skeletal muscle. Thus, pre-synaptically, the α-action on motor nerve terminals increases the amount of acetylcholine released in skeletal muscle, but reduces it in the sympathetic ganglion. However, there is still some controversy on this point and further investigation is required to determine the conditions of the tissue which favour potentiation or inhibition. The post-junctional inhibition is a β-effect in skeletal muscle, but an α-effect in the sympathetic ganglion. This poses another important question, i.e. what determines the direction of the response to the α- and β-components?

Bowman & Raper (1966) pointed out 'that it is the nature of the tissue rather than the side of the junction which determines the response'. They thought that hyperpolarization of nervous tissue might always be an α-effect, and hyperpolarization of mucle tissue always a β-effect. But, once more, this generalization is not the whole answer.

Smooth muscle

Both α- and β-receptors are present in almost every smooth muscle. The β-effect is always inhibition, but in many smooth muscles the β-inhibition is not associated with hyperpolarization. On the other hand, the α-effect in some muscles is inhibitory—especially in the gut—and this inhibitory α-effect is usually associated with hyperpolarization. In other smooth muscles the α-effect is excitatory and is associated with depolarization.

What then determines the response?

Bowman & Nott (1969) put forward an explanation for the difference between the responses of fast and slow skeletal muscles to catecholamines. They suggested a β-effect—perhaps via cyclic AMP—on the rate of calcium release from and calcium reuptake into intracellular stores. In slow muscle the reuptake of calcium into the sarcoplasmic reticulum might be accelerated, hence the fast rate of relaxation in contrast to the fast muscles in which the reuptake might be suppressed and the contraction prolonged.

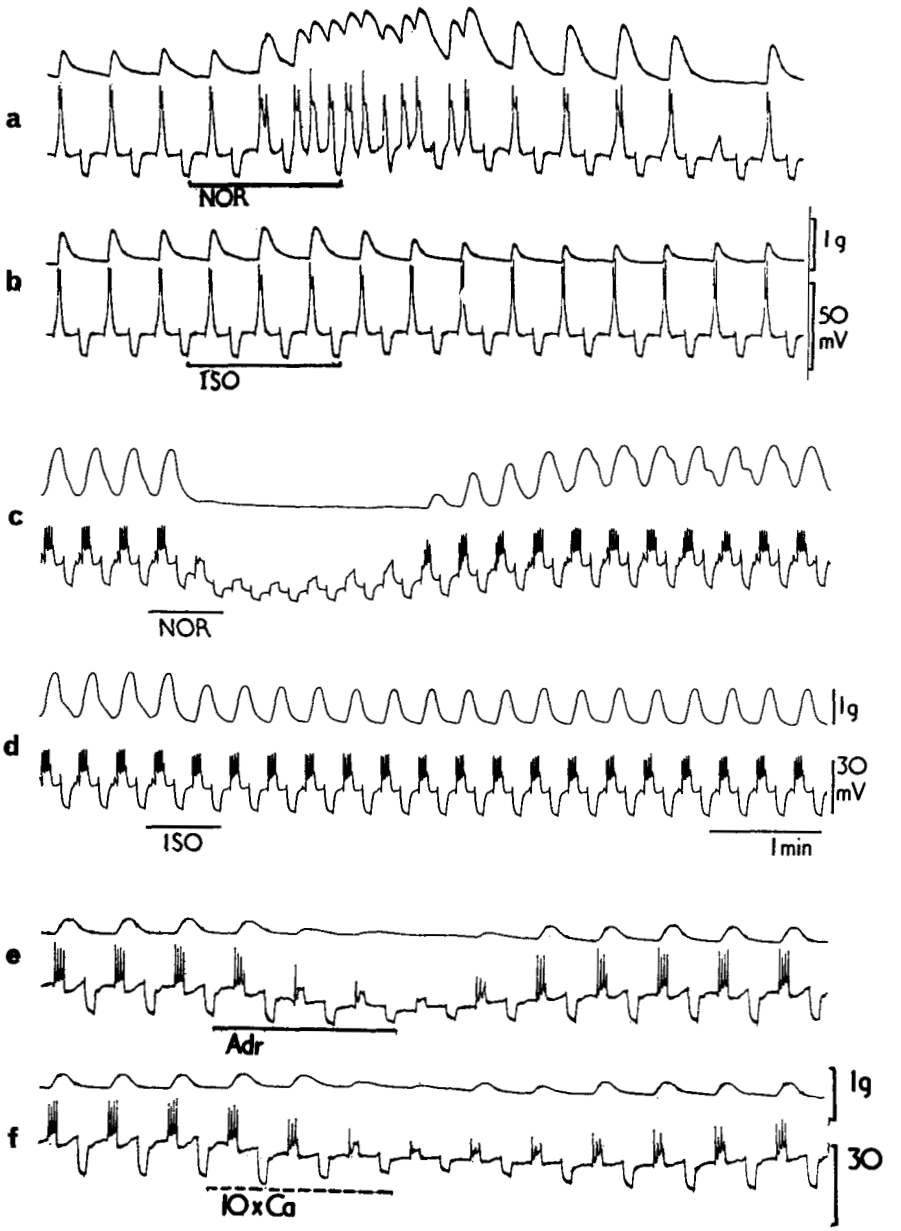

FIG. 5. The α- and β-effects of catecholamines on guineapig-uterus and taenia coli. Double sucrose gap method. In all records the upper trace shows the mechanical, the lower trace the electrical responses to constant current pulses of alternating polarity. (a) Oestrogen + progesterone dominated uterus. Excitatory α-effect of noradrenaline (10^{-5} M) applied for 1 min (bar) producing depolarization, repetitive firing and maintained contraction; (b) Inhibitory effect of isoprenaline (10^{-5} M) causing no change in membrane activity, but smaller contractions (from Bülbring & Hardman, 1976, with permission); (c) Taenia coli. Inhibitory α-effect of noradrenaline ($1{\cdot}75 \times 10^{-7}$ M) applied for 1 min (bar) producing a decrease in membrane resistance and hyperpolarization; (d) Inhibitory β-effect of isoprenaline ($2{\cdot}25 \times 10^{-7}$ M) causing no change in membrane resistance and membrane potential, but smaller contractions (from Bülbring & Kuriyama, 1973, with permission); (e) Taenia coli. The inhibitory α-effect of adrenaline (2×10^{-7} M) (in the presence of propranolol 2×10^{-6} g ml^{-1}) is mimicked (d) by the effect of increasing the external calcium-concentration 10 times for 1 min.

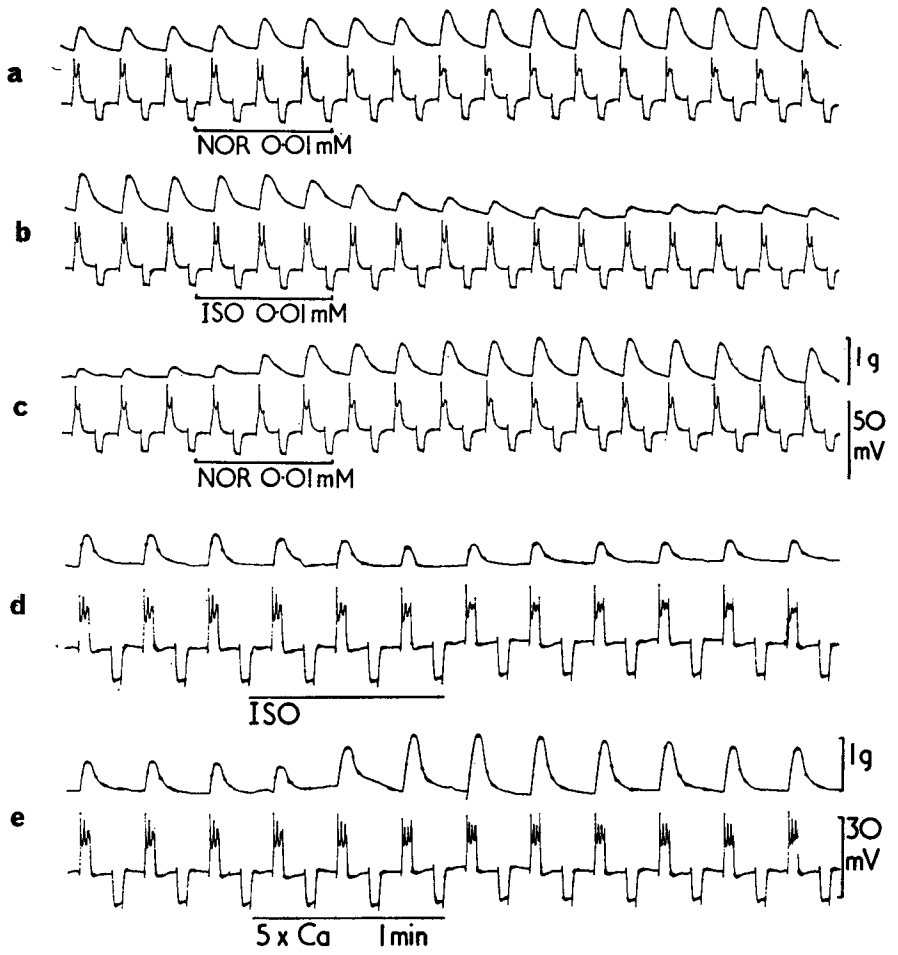

FIG 6. Effect of catecholamines on guinea-pig uterus (oestrogen + progesterone dominated). Double sucrose gap method. Records as in Fig. 5. (a) Potentiation of muscle contractions by noradrenaline; (b) reduction by isoprenaline, and (c) increase by second application of noradrenaline (adapted from Bülbring & Hardman, 1976, with permission); (d) prolonged inhibitory β-effect of isoprenaline (1×10^{-6} M) in the presence of theophylline (5×10^{-4} M) is antagonized (e) by a 5 times increase of the external calcium concentration for 1 min (adapted from Bülbring, 1973a, with permission).

As far as I know, the β-action on all smooth muscles would then resemble that of the slow skeletal muscle, since the β-action reduces the size of contractions (Fig. 5b, d; Fig. 6b, d). This may well be due to the reduction of the intracellular calcium ion concentration caused by accelerated calcium uptake into stores. On the other hand, much recent evidence suggests that the α-action of catecholamines causes calcium release from internal stores. One internal calcium store is the sarcoplasmic reticulum, which is developed to different degrees in different muscles. Another storage site is the binding of calcium to the inner surface of the cell membrane where it seems to control the potassium permeability of the membrane and hence the membrane potential. One theory is that the turnover of this internally membrane-bound calcium, i.e. its periodical removal and reuptake, is the basis for rhythmic spontaneous activity (Tomita & Watanabe, 1973).

The degree and the pattern of spontaneous activity varies in different smooth muscles and is correlated with the level of the resting membrane potential. The main factor controlling this is the potassium permeability of the membrane and this, as already mentioned, depends on the calcium binding capacity at the inner surface of the membrane. In the taenia coli, which is continuously spontaneously active, the resting membrane poten-

tial is seldom reached and the amount of calcium bound at the inside of the membrane is probably very low. In other smooth muscles, e.g. the uterus, the membrane potential is high, presumably because the amount of calcium bound on the inside of the membrane is high, and the binding sites may be saturated.

When now, in response to the the α-action of catecholamines, the cytoplasmic calcium ion concentration is suddenly increased—be it by calcium-entry and/or by calcium-release—then it will depend on (a) the availability of calcium-binding sites just inside the membrane and (b) on the development of the sarcoplasmic reticulum whether the dominant result will be (a) calcium-binding to the membrane (as we believe happens in the taenia), or (b) calcium-activation of the contractile protein (as we believe happens in uterus).

The observations on the two tissues are consistent with this hypothesis:

The α-effect on the taenia is inhibitory, because the hyperpolarization, caused by the increased potassium conductance, leads to block of spike activity and relaxation (Fig. 5c). The β-effect on taenia is also inhibitory, but the reduction in the size of contractions occurs without detectable change of membrane potential, membrane resistance or electrical activity (Fig. 5d).

The α-effect on the uterus is stimulant. The membrane depolarization, mainly due to increased chloride-conductance, leads to repetitive firing and a maintained contraction (Fig. 5a). The β-effect, as in taenia, is a reduction of the size of contractions (Fig. 5b).

Some of the evidence for the involvement of calcium is that the α-effect, whether inhibitory or excitatory, is mimicked by excess calcium, while the β-effect is antagonized by excess calcium. Fig. 5e, f shows that, on taenia, the effect of 1 min exposure to high external calcium mimics the inhibitory α-effect of 1 min exposure to adrenaline.

The α-effect on individual uterine contractions can be most clearly demonstrated with threshold concentrations of noradrenaline which produce no membrane excitation. In Fig. 6a, b, c, the contractions evoked by constant depolarizing current pulses are increased by noradrenaline, depressed by isoprenaline and restored by a second application of noradrenaline, showing the antagonism between α- and β-action, without detectable effects on membrane activity. A similar antagonism is illustrated in Fig. 6d, e, in which the β-action of isoprenaline is antagonized by brief exposure to a high external calcium concentration.

That the α-excitation of the uterus is mimicked by excess calcium, and is abolished by calcium-removal, is not surprising. But that the increase in potassium-conductance, which is the essence of the α-inhibition in the taenia, is also mimicked by excess calcium, that it is abolished by calcium-removal and restored by re-admission of calcium, lends support to the interpretation that calcium is essential for the increase in potassium-conductance, causing hyperpolarization. Moreover, the strategic site at which the presence of calcium brings about the α-action in the taenia coli is thought to be at the inner surface of the cell membrane. Fig. 7 illustrates the hypothesis which is based on strong, but only indirect, evidence in the form of a diagram. Obviously, the next step in the analysis of the mechanism of the action of catecholamines must be the localization of calcium, the study of subcellular calcium distribution and, perhaps, the demonstration of a calcium-translocation inside the cell in response to the α- and β-action.

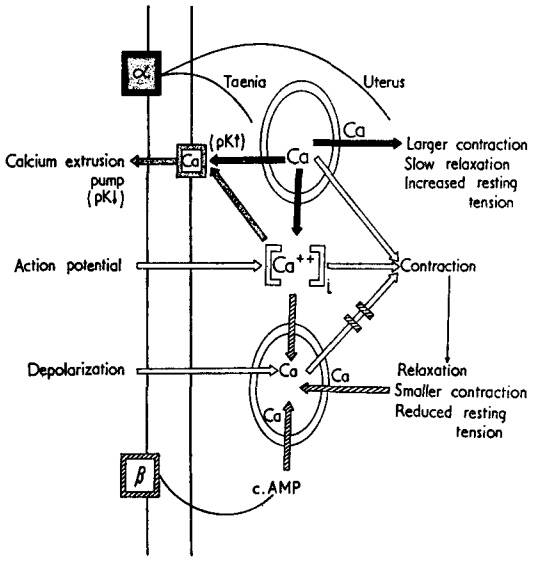

FIG. 7. Diagram of the possible mechanism of action of catecholamines on smooth muscle. α- and β-receptors are shown on the outside of the cell membrane. Calcium-binding site (stippled) on the inner surface of the membrane. The calcium ion concentration of the cytoplasm, $[Ca^{++}]_i$, is determined, firstly, by the balance of calcium-entry during activity and calcium-extrusion, secondly, by calcium-release from and calcium-uptake into the sarcoplasmic reticulum (ovals). The α-action (black) and β-action (striped) influence intracellular calcium-translocation as indicated (from Bülbring, 1973b, with permission).

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