EFFECT OF DRUGS ON SMOOTH MUSCLE[1,2]

By G. Burnstock and M. E. Holman
Department of Zoology, University of Melbourne, and
Department of Physiology, Monash University,
Victoria, Australia

I. INTRODUCTION

The interpretation of the results of pharmacological experiments on smooth muscle has been very difficult because of the lack of precise knowledge of three factors:

(*a*) The morphology and physiology of smooth muscle cells and their innervation.
(*b*) The mechanisms of uptake, synthesis, storage, release, and inactivation of transmitters from autonomic nerves.
(*c*) The mode of action of drugs on the smooth muscle cell membrane.

The progress that has been made during the past few years in interpreting the effects of drugs on smooth muscle has largely been achieved through investigations of these factors. However, in general, the results have only served to emphasize the complexity of the problem, and the inadequacy of many of the earlier explanations.

In this review we will not attempt to discuss the experiments of drug action on organs *in vivo* where interpretation is complicated by further factors such as central nervous control, circulating hormones, and secondary cardiovascular effects. We will confine the discussion to electrophysiological analysis of drug action at the cellular level. In Section II, the electrical responses of smooth muscle cells to the direct application of drugs will be described in terms of ionic conductances. In Section III, the effects of drugs on specific processes such as transmision of excitation and inhibition from autonomic nerves to single smooth muscle cells will be discussed.

The Innervation of Smooth Muscle

There is now strong evidence from electron microscope studies (133, 143, 166–168) and from fluorescent histochemistry (84, 153) to support and extend the earlier hypotheses of Rosenblueth (172) and Hillarp (109). It appears that the autonomic innervation apparatus consists of long varicose nerve fibres (about 15 to 30 varicosities/100 μ) which run in bundles in parallel with the longitudinal axis of the muscle fibres. The axons are

[1] The survey of the literature pertaining to this review was concluded in April 1965.

[2] The following abbreviations will be used: DOPA (3,4-dihydroxyphenylalanine); DOPAmine (3,4-dihydroxyphenylethylamine); ACh (acetylcholine); TEA (tetraethylammonium); IAA (iodoacetate); DNP (dinitrophenol); RMP (resting membrane potential); EJP (graded excitatory junction potential); SEJP (spontaneous excitatory junction potential); IJP (inhibitory junction potential).

for the most part enclosed by Schwann sheath and terminate as swollen endings in shallow grooves in muscle cells. The varicosities contain numerous vesicles and mitochondria and high concentrations of transmitter. Transmitters are probably released from these varicosities, to form "en passage" junctions, as well as from the nerve endings. The separation of pre- and postjunctional membranes at nerve endings and at a small number of the "en passage" junctions is about 200 Å. The muscle membrane in these regions does not appear to have a specialized structure. Evidence for the release of transmitter from "en passage" junctions over considerable lengths of the varicose regions of autonomic nerves is provided by electrophysiological observations of the transmission process [(49–52, 93, 127), see below], and has also been suggested from measurements of the amounts of transmitter liberated from the nerves (27). Furthermore, recent studies with fluorescent histochemical methods have shown that varicosities of the nerve fibres take up or release norepinephrine (138).

There is considerable variation in the proportion of varicosities with separations from the muscle membrane of greater than 200 Å to close junctions with 200 Å separation in different tissues. The rat vas deferens, for example, appears to have a high proportion of junctions with a separation of 200 Å, with probably one or more to every muscle cell (166–168). On the other hand, the guinea pig vas deferens has relatively fewer close junctions compared to the number of more distant varicosities (143). In the gut, close junctions are rarely seen, and the majority of nerves run in bundles with more than 500 Å separation of varicosities from the muscle cells (91, 102, 103, 165, 188, 201, 202), suggesting that in this case the transmitter reaches most muscle cells diffusely from many distant sources. It is interesting to note that when close (200 Å) junctions have been seen in the intestine, they are often "multiaxonal" (26, 170), i.e., several axon profiles containing vesicles lie in close apposition to a single muscle fibre. In most vascular smooth muscles there is a dense nerve plexus which is confined to the inner surface of the adventitia and nerve fibres rarely penetrate into the medial muscle layer (8, 56, 70, 84, 101, 136). It is likely that the degree of interaction between neighbouring smooth muscle cells is related to the density of innervation (56, 58, 76, 82, 155). This concept of the autonomic end apparatus suggests hat there may be more extensive prejunctional sites for drug action than at the skeletal neuromuscular junction where transmitter release is confined to nerve endings.

Transmitter Mechanisms

There are a number of recent reviews describing the biochemistry of the synthesis, uptake, storage, release, and inactivation of the transmitters of autonomic neurons (5, 17, 80, 122, 139, 183). The adrenergic neuron is capable of actively taking up and storing catecholamines (19, 77, 81, 95, 104, 107, 144, 160, 171, 199). The suggestion has been made that the norepinephrine released from nerves may then be reabsorbed by them, and that this uptake mechanism may determine the time course of transmitter

inactivation (122). Some drugs (e.g., cocaine) appear to act indirectly by interfering with this mechanism of uptake of catecholamines (173, 192). Other drugs (e.g., nicotine, guanethidine, amphetamine, ephedrine, tyramine, morphine, monoamine oxidase inhibitors) cause increase or decrease of release of norepinephrine (16, 18, 62, 66, 145, 162, 191). Yet further drugs (e.g., DOPA, DOPAmine, hemicholinium, and α-methyl-*m*-tyrosine) act indirectly by increasing or preventing the synthesis of transmitter (67, 97, 180, 200). The prejunctional action of drugs is complicated by the suggestion that norepinephrine is present in two pools, one labile, the other bound (194). Several binding sites for norepinephrine in the nerves have been postulated (123, 163, 194).

Evidence has been presented that acetylcholine (ACh) is involved in adrenergic transmission, and various explanations have been put forward to account for this (39, 87, 121, 135).

The Effector Cell

Zaimis (203) warns: "Recently there has been a growing tendency to represent the pharmacological effects of drugs acting on structures innervated by adrenergic nerves as if they took place exclusively at the nerve endings, in other words, as if the effector cells played a minor role or none at all. . . . Such systems are unrealistic." However, when considering the postjunctional action of drugs, the role of the innervation in the particular tissue being examined must be taken into account.

By analogy with the skeletal neuromuscular junction (79), it seems likely that the sensitivity of the smooth muscle membrane in the region of a close junction (200 Å) may be considerably higher than the rest of the membrane. In contrast, the sensitivity of the membrane of muscle cells receiving transmitter by diffusion from many distant sources may be homogeneous. Bennett (unpublished data) has recently shown that transmural stimulation of the guinea pig taenia produces an inhibitory response in most cells, while other cells less than 0.5 mm away give an excitatory response. These findings can be explained in terms of the position of the cells in relation to the concentration of different transmitters reaching the cell from sources at variable distances. An asymmetry of the spatial arrangement of nerves might lead to differences of sensitivity of different smooth muscle cells to different transmitters.

There are now many reports of the lack of specificity of blocking drugs on postjunctional as well as on prejunctional sites (23, 124, 151). For example, phenoxybenzamine, phentolamine, and guanethidine antagonize acetylcholine as well as blocking responses to adrenergic nerve stimulation and to applied norepinephrine (23). Atropine and xylocholine have been shown to block the action of catecholamines in addition to their other actions (37, 105, 108, 198). It has been suggested that there may be a similarity between adrenergic and cholinergic receptors in their affinities for blocking drugs such that pharmacological differentiation is difficult (23, 57). Both α- and β-adrenotrophic receptors have been demonstrated in the same smooth

muscle (1, 2, 89, 112, 134, 174, 186, 193) although it is not known yet whether these receptors are confined to different cells or whether both are present on each cell. The species differences in receptor sensitivity to drugs and in the types of receptors present deserve fuller consideration (196).

An Example of Difficulties in Interpretation of Drug Action

The problems involved in interpretation of the action of drugs on whole organs, which have been stressed so far, may be exemplified by consideration of a smooth muscle system with a complex innervation, such as the intestine (125, 197). The action of drugs on ganglion-free circular intestinal muscle has been studied (83, 137, 164), but this of course does not mean nerve-free. The concentrations of local anaesthetics which are required to block conduction in these nerves also affect the smooth muscle directly (46). Cold storage of isolated preparations for several days may cause degeneration of most nerves, but it may also cause irreversible changes to the smooth muscle. Although it is possible surgically to denervate so that the extrinsic sympathetic and parasympathetic nerves of the intestine degenerate, it is impossible to remove all the intramural neurons.

Some drugs may act indirectly by releasing pharmacologically active substances from stores in argentaffin, enterochromaffin, or mast cells in the wall of the gut (85, 106, 146).

Drugs applied to an *in vitro* preparation of intestine may act at a number of neuronal sites. Apart from the prejunctional action already mentioned, they may act at the ganglion synapse, either to stimulate the ganglion cell or to block synaptic transmission. It is well known that drugs such as nicotine first excite, then block ganglia, so the time course of diphasic action of this kind must be taken into account. The recent observation of noradrenergic terminals on cell bodies in Auerbach's plexus (152) extends this problem of drug action to include the question of whether the action is on cholinergic or adrenergic mechanisms in such synapses. At least two types of ganglion cells are present in Auerbach's plexus, including excitatory and inhibitory neurons (4, 46). The existence of intramural neurons in Auerbach's plexus, which are inhibitory but differ from the sympathetic nerves, has been demonstrated electrophysiologically (12, 13, 44, 45). The suggestion has been made that the intramural inhibitory nerves are responsible for the descending inhibition during peristalsis, and that their connections with the central nervous system are through parasympathetic pathways (46, 61). They may also be involved in the inhibition of the intestine caused by local distension (111). Intramural excitatory neurons are probably cholinergic (28, 125). Drugs may act on either or both excitatory or inhibitory neurons, and the effects produced may be determined by the relative numbers of each type of neuron stimulated. The concentration of a drug which acts on both types of neuron must be considered. For example, weak concentrations of nicotine cause relaxation of the rabbit gut, whereas high concentrations produce contraction (97). Neostigmine reverses the response to transmural stimulation of the gut from relaxation to contraction,

probably by altering the balance in favour of cholinergic nerve responses in competition with inhibitory nerves (9, 45, 177).

There has been much debate through the years whether the continuous spontaneous release of acetylcholine in the gut is of a nervous or non-nervous origin (3, 83, 86, 117). However, regardless of the origin, drugs such as eserine and neostigmine act indirectly on the gut by potentiating the effects of spontaneously released acetylcholine (40). Atropine depresses spontaneous activity in the fish gut, and this is probably caused by antagonism of the action of the spontaneously released acetylcholine (40).

It has been claimed on the basis of fluorescent histochemical staining that sympathetic adrenergic fibres supplying many visceral organs do not, as was previously thought, run directly to the smooth muscle layers, but are mainly distributed to the blood vessels or to intramural ganglia (153). Thus, transmitters may be released not only from nerves directly supplying the gut musculature, but also from the sympathetic nerve plexuses supplying the vascular smooth muscle in small blood vessels and arterioles running between muscle bundles and in the submucosa. Considerably more physiological evidence is needed before this claim can be substantiated.

The sensory elements of the peristaltic reflex arc described by Bülbring, Lin & Schofield (36) may be yet another site of drug action. Drugs reaching the mucosal surface of a segment may stimulate sensory endings located at the bases of columnar epithelial cells, or they may act on sensory ganglion cells in Meissner's plexus.

Many of the earlier accounts of drugs applied to gut segments causing inhibition followed by contraction were interpreted as actions on both inhibitory and excitatory elements. The recent work from our laboratory (10, 44, 61) has shown that this diphasic action can be explained in terms of "after contractions" due to rebound or "anodal break exaltation" (7) of the smooth muscle membrane following the hyperpolarizing action of transmitters or drugs causing relaxation.

The response to a drug depends on the initial tone of the preparation: low tone favours excitation and high tone favours inhibition (148). Under some conditions (e.g., when a low tone is present), an inhibitory drug may not cause a relaxation.

The results described earlier, that different muscle cells of the taenia give either an excitatory or inhibitory response upon stimulation of the intramural nerves, bring up the possibility that drugs act differently on different muscle cells in the population. Certainly there is no real basis for the assumption that all muscle cells in a segment of gut are equally sensitive to a drug.

II. EFFECT OF DRUGS ON THE SMOOTH MUSCLE CELL MEMBRANE

Most studies on drug action at the membrane level, which will be discussed in this section, have been carried out on the spontaneously active smooth muscle—notably the estrogen-dominated rate uterus and the guinea

pig taenia coli. Although this activity *in vivo* may be under the control of extrinsic and intrinsic nerves and circulating hormones, there is little doubt about its myogenic origin (55).

Drugs which change the pattern of spontaneous activity in isolated preparations are probably acting directly on the smooth muscle membrane. However, it is always possible that part of their action may be caused by the release of neurohormones from local stores or from autonomic nerve terminals.

Studies on the effects of drugs on the activity of smooth muscles in response to electrical stimulation are even more likely to be confused by the action of the drug at sites other than the smooth muscle cell membrane. The response of many preparations to transmural or "field" stimulation is likely to be mainly a result of the activation of intramural axons. For example, the response of the taenia coli to "field" stimulation may be caused by the excitation of either inhibitory or excitatory nerves or by a combination of the two. Drugs which alter the response of the taenia coli to electrical stimulation will be discussed in Section III.

There are some smooth muscles, however, in which the excitation of intramural nerves probably does not play a significant part in the response to transmural stimulation, at least if single pulses are used. These muscles include the uterus (68) and the ureter of various species (55, 120). Drugs which affect the configuration of the action potentials of these muscles in response to electrical stimulation are probably acting directly on the smooth muscle membrane.

The direct excitation of the smooth muscle cells by the passage of a depolarizing current from an intracellular electrode has been achieved for the taenia coli (131, 132) and the guinea pig vas deferens (113). In these experiments, and also in those on cat intestine (150, 182), cell resistance has been measured by a bridge technique in which a single electrode passes current while monitoring the membrane potential. Although it is difficult to deduce absolute values for conductances of the smooth muscle cell membrane, owing to uncertainties about its dimensions, this method provides a means of determining changes in conductance caused by drug action. If changes in conductance are determined when the muscle is exposed to different ionic environments, it should be possible to work out the details of drug action at the membrane level in terms of specific changes in conductance for Na, K, and Cl ions.

Ionic Basis of Membrane Potential in Smooth Muscle

Most of the current theories of drug action at the cellular level have been framed in terms of the ionic hypothesis of Hodgkin & Huxley (110). A full account of the status of this theory in explaining the membrane phenomena of smooth muscle is beyond the scope of this review, although this question has been much discussed recently (33, 55, 65, 120, 126, 129, 141, 175).

At the present time it is by no means certain that we are justified in

assuming that the principles which apply to nerve and skeletal muscle, also apply to smooth muscle. Individual ionic conductances (g_{Na}, g_K, and g_{Cl}) have yet to be measured in smooth muscle, and there are still many uncertainties about the values of the equilibrium potentials E_{Na}, E_K, and E_{Cl}. Nevertheless, most of us working in this field have accepted the ionic theory in general terms as a working hypothesis. Before some of the current theories of drug action at the membrane level are considered from this point of view, it may be useful to survey some recent experiments in this field.

The difficulties involved in determining the intracellular concentrations and, hence, the equilibrium potentials for Na, K, and Cl ions have been summarized by Bohr (20) and Burnstock, Holman & Prosser (55). The total Na content varies from one smooth muscle to another within the range of 60 to 120 m*M*/kg wet weight (55, 65, 88, 118).

If the tissue Na is partitioned between the somewhat arbitrary volume of the extracellular space and the intracellular water (Na_{in}) usually turns out to be higher than that of most other excitable tissues, and E_{Na} is therefore low. For example, Casteels & Kuriyama (65) found values for E_{Na} of around +24 mV when Na was partitioned according to inulin space. Much higher values of E_{Na}, approximating those of the skeletal muscle, were calculated on the basis of ethanesulphonate space (+50 to +60 mV).

It is difficult to understand how smooth muscles can be in osmotic equilibrium if (Na_{in}) is as high as some authors have proposed (88). However, smooth muscles do not always behave as simple osmometers (25). Since the cells are of a small diameter (less than 5 μ) they have a relatively large surface area to volume ratio, compared with other muscles. It may turn out that the composition and properties of the extracellular space are important in determining both electrolyte distribution and osmotic phenomena.

Most of the tissue Na exchanges very rapidly (47, 99). Concurrently, measured efflux curves for I^{131} labeled albumin and Na^{22} show that part of the rapidly exchanging fraction must be intracellular, or else it must be accumulated in the extracellular space (47). Whether or not this rapid exchange of Na is associated with a high resting Na conductance (g_{Na}°) remains to be seen. It is possible that Na (and Cl also) may enter and leave the cell by a process which does not contribute to membrane conductance. The large number of micropinocytotic vesicles on the surface membranes of smooth muscle cells suggest there may be a continuous exchange between interstitial space and cell cytoplasm (55).

Evidence for this active transport of Na will be discussed later in relation to the action of metabolic inhibitors. Since the work of Bozler (24), many authors have agreed that both the membrane potential and excitability may be more closely coupled to cell metabolism in smooth muscle than in other excitable tissues (29, 31, 32, 55, 142). Since the surface area-to-volume ratio is large, it is conceivable that this coupling might be caused by changes in ionic distribution, because of changes in the activity of a

Na-K-linked active transport system. On the other hand, an electrogenic Na pump, like that of frog skin, has also been proposed by Burnstock (42). These ideas have been debated by Bülbring, Kao, Daniel, and others (32, 74).

The total tissue potassium content is low, and E_K turns out to be 80 to 95 mV—probably 15 to 25 mV higher than the resting membrane potential (RMP) (20, 55, 65, 118, 140). Intracellular Cl is high (64, 98, 118). Casteels found a value of −26 mV for E_{Cl} in the taenia coli. The high value of intracellular Cl appears to be due to the active uptake of Cl (64). This is blocked by low external K, low Na, low temperature, DNP, and ouabain. Casteels suggests that active Cl transport may be linked to the Na-K exchange in this tissue.

Most smooth muscles have RMP's of 55 to 75 mV (55). These values are low compared with many striated muscles. It is possible that the difference between the resting Na conductance ($g_{Na}°$) and K conductance ($g_K°$) may be less marked than in skeletal muscle. A lower $g_K°/g_{Na}°$ ratio would help to explain the relationship between the RMP and changes in $(K)_o$ observed in smooth muscles (55, 126, 129, 140). Bülbring and her colleagues (29, 65, 126, 129) consider that the smooth muscle membrane has a relatively high value of $g_{Na}°$ rather than a low $g_K°$. Evidence for a high $g_{Na}°$ has also been found for spontaneously active cardiac Purkinje fibres (190). A high $g_{Na}°$ may be characteristic of all tissues which tend to be spontaneously active.

The spontaneous activity of Purkinje fibres appears to be caused by the combination of a high $g_{Na}°$ together with a time-dependent decrease in $g_K°$ during diastole. Measurements of membrane conductance during changes in ionic environment suggest that g_K is high at the beginning of the diastole and steadily decreases until the Na current exceeds the K current and a new action potential is generated (190). Thus, diastolic depolarization (the pacemaker potential) is dependent on the preceding action potential. Some of the action potentials encountered in well stretched preparations of taenia coli, or during trains of action potentials from pacemaker regions of the uterus, show a close resemblance to those of cardiac pacemakers. It is tempting to think that the ionic basis of spontaneous activity in these tissues may be similar. However, it is clear that this explanation cannot account for all the patterns of spontaneous activity recorded from smooth muscles.

One of the outstanding problems of smooth muscle physiology concerns the origin and nature of the slow waves of depolarization which determine the pattern of its mechanical and electrical activity (21, 22, 55, 149). The slow waves of intestinal muscle consist of rhythmic fluctuations in membrane potential which decrease in frequency from duodenum to terminal ileum. They are the most prominent features of records taken with external electrodes and appear to be synchronous over large numbers of smooth muscle cells. They do not depend on the presence of action potentials and

occur in the absence of any mechanical changes. Their amplitude can be varied by a number of drugs and ions (55), but these agents do not usually change their frequency. The familiar student experiments on rabbit ileum illustrate these points well. The frequency of "beating" is unaffected by changes in K, Ca, or Na concentrations, by small doses of acetylcholine and epinephrine, or by changes in length or tension, but is very sensitive to temperature. Although it is important to remember that there may be fluctuations in the excitability of the smooth muscle membrane, which are not associated with changes in membrane potential (65, 140), it seems likely that the slow waves reflect the basic mechanism which sets the pattern of activity of any particular organ. They are probably linked to cellular metabolism as predicted by Bozler (24). Their ionic basis is unknown.

The effect of reducing the external Na concentration on the amplitude and rate of rise of action potentials depends on the substance that is used to replace Na or NaCl (33, 55, 68, 75, 120, 130, 140). In sucrose solutions, action potentials are abolished in most smooth muscles unless a critical amount of Na is present (10 to 20 m*M*). Csapo & Kuriyama (68) suggest that this might be because of a "relatively moderate change in E_{Na} due to the rapid decrease in Na_{in}, followed by a change in Na_{out}." The high threshold and slow rate of rise of smooth muscle action potentials suggest that the g_{Na} mechanism may be saturated at relatively low Na concentrations (i.e., there may be relatively few "Na carriers" available). This idea is supported by the observation that excess Na does not greatly increase the rate of rise or overshoot of the action potential (33). A relatively small increase in g_{Na} during depolarization may be an intrinsic property of the smooth muscle membrane or, alternatively, the g_{Na} mechanism may be inactivated by the low RMP which is characteristic of this tissue.

Ca ions have been found to influence the action potential mechanism of smooth muscle in much the same way as Ca acts in other tissues (55). There have been several suggestions that membrane phenomena in smooth muscle are dependent on the binding of Ca by the cell membrane (29, 100). Bülbring & Kuriyama's theory that both $g_{Na}{}^{o}$ and g_{Na} are dependent on the degree of fixation of Ca by the cell membrane will be discussed in the next section in relation to the inhibitory action of epinephrine.

The Inhibitory Action of Epinephrine

A number of attempts have been made to account for the inhibitory action of epinephrine in terms of its effects on individual ionic conductances. Since g_{Na}, g_K, and g_{Cl} have yet to be measured, it has been necessary to make deductions from the changes in the response to drugs when smooth muscles are exposed to various different ionic environments. As a result of a series of experiments of this kind on the taenia coli, Bülbring & Kuriyama (34) have confirmed the previous suggestion of Burnstock (42) that the inhibition of spontaneous activity by epinephrine is brought about by changes in Na conductance. They suggest that both $g_{Na}{}^{o}$ and the increase in g_{Na} during depo-

larization are determined by the degree of Ca fixation on or near the smooth muscle cell membrane. Under normal conditions, Ca fixation is assumed to be poor. Smooth muscle cells therefore have a low and unstable membrane potential. Action potentials are characterized by slow rates of rise and insensitivity to external Na concentrations. Bülbring & Kuriyama propose that epinephrine increases the fixation of Ca in the membrane and that this results in stabilization of the membrane potential, hyperpolarization, an increased rate of rise of the action potential, and other changes resembling the effects of increased Ca [see Shanes (176)]. Since the action of epinephrine on the taenia coli is blocked by exposure to glucose-free media and cold temperatures, Ca fixation is likely to be dependent on metabolic processes which require energy (9).

According to this theory, the inhibitory action of epinephrine should be associated with a decrease in membrane conductance. Kuriyama (personal communication) found evidence for such a decrease, and it is important that these experiments are repeated, using intracellular electrodes to pass current and measure membrane potential directly.

Epinephrine increases the amplitude and the rates of rise and decay of the action potentials in the taenia coli. In this respect its action is identical with that of excess Ca (29, 34). Excess Ca, however, does not cause relaxation of this smooth muscle, since it does not block the generation of action potentials. Thus, epinephrine differs from excess Ca in its specific effects on the mechanism involved in the generation of action potentials.

Epinephrine can block spontaneous activity in smooth muscles such as the taenia coli and rat uterus without necessarily causing an increase in membrane potential. The duration of the period of cessation of active potentials depends on the concentration of epinephrine (35, 68). In most preparations, some hyperpolarization accompanies the slowing and cessation of action potentials. After action potentials have been blocked, the membrane potential of the taenia coli may undergo subthreshold fluctuations which appear to be of at least two types (35). Slow variations in membrane potential ("slow waves") occur, which have a similar time course to the prepotentials of the pacemaker type which, under normal conditions, would generate action potentials. Superimposed on these slow fluctuations are briefer depolarizations whose overall duration is similar to that of an action potential. It seems likely that these brief depolarizations are either abortive action potentials or electrotonic potentials because of action potentials arising in neighbouring regions not yet blocked by epinephrine. Similar potentials were observed by Bennett, Burnstock & Holman (12) during submaximal stimulation of the perivascular sympathetic nerve supply to the taenia coli (in the presence of atropine). Records of this type clearly illustrate the stabilizing action of epinephrine on the smooth muscle cell membrane. Epinephrine also has a stabilizing action on rat myometrium under estrogen or progesterone influence. Both the threshold for firing and the amplitude of action potentials increase in the presence of epineph-

rine (68). Prepotentials of the pacemaker type are prolonged, but there is a decrease in the number of action potentials in each train.

Epinephrine causes the immediate cessation of activity in spontaneously active preparations of chick amnion, although slow complex fluctuations in membrane potential are maintained for some minutes after mechanical activity has ceased (69). When action potentials return after washing out, they are increased in amplitude.

The degree of hyperpolarization produced by epinephrine depends on the level of the resting membrane potential; the lower the RMP the greater is the hyperpolarization. This is so if the RMP is reduced by stretching the preparation (35) or if the RMP is reduced by ACh (34). The ionic basis of these observations may be that epinephrine causes an increase in the ratio g_K^o/g_{Na}^o. ACh probably causes an increase in both g_{Na}^o and g_K^o (34), and potentiation of the effect of epinephrine would be expected to occur (see below). The action of epinephrine on a preparation depolarized by ACh is in marked contrast to the action of epinephrine in the presence of excess K. In K concentrations greater than 30 m*M*, hyperpolarization in response to epinephrine is markedly reduced or completely blocked, although there may still be an inhibition of spontaneous activity. In a K-free solution, epinephrine again gives rise to a large hyperpolarization (34).

In most excitable tissues, g_K^o increases as the external K concentration is increased (176). In high K concentrations, the RMP approaches E_K. According to Kuriyama (126), the relationship between extracellular K concentration and RMP in the taenia coli differs from that predicted by the Nernst equation unless the external Na concentration is reduced and the external Cl is replaced by an impermeable anion such as SO_4. His results show that although the RMP diverges from E_K at all K concentrations, this divergence is increased when K concentration is reduced. Thus, an increase in g_K^o/g_{Na}^o would be expected to cause a much greater increase in membrane potential in low K concentrations than in high K concentrations.

Excess Na reduces the hyperpolarization and depresses but does not usually block the inhibitory action of epinephrine. Excess Na itself causes depolarization of the smooth muscle cell membrane resulting, perhaps, from a significant contribution of g_{Na}^o to total resting membrane conductance. A decrease in g_{Na}^o or an increase in g_K^o should cause a large hyperpolarization under these conditions, and it is difficult to explain why this does not occur. In excess Na solutions, large prolonged local potentials are observed (34) which generate bursts of action potentials. Epinephrine may depress these "generator potentials," but it does not prevent them from initiating action potentials.

Reduction of Na to 50 per cent has no effect on the action of epinephrine, but in Na-free solution (replacement with Tris-Cl or Li Cl), the action of epinephrine is reduced or abolished. This observation has been taken as

evidence that the action of epinephrine is caused by a specific decrease in g_{Na}° rather than an increase in g_K° (34). However, if the smooth muscle membrane behaved in accordance with the classical ionic hypothesis, blockade of epinephrine hyperpolarization by low or zero Na solutions could equally well be accounted for if epinephrine caused an increase in g_K° rather than a decrease in g_{Na}°. It has been pointed out that the ionic basis of the electrical activity that persists in low or Na-free solutions is far from clear, and it is difficult, therefore, to draw any conclusions from the action of epinephrine under these conditions.

It has long been known that, although epinephrine inhibits the action potentials which trigger contractions in other intestinal preparations, it does not block the rhythmic slow waves which normally generate the action potentials (*22*, 55). In the taenia coli, local potentials analogous with the slow waves of the small intestine are not always readily distinguished from other subthreshold fluctuations in membrane potential. It is possible that epinephrine might block the action potential mechanism in the taenia without depressing the local potential from which it normally arises.

Bueding & Bülbring (*29*) have summarized the evidence that the action of epinephrine is dependent on an increase in cell metabolism leading to an increase in synthesis of high energy compounds including cyclic AMP, ATP, and creatine phosphate. They have emphasized the similarity of the inhibition by epinephrine with that caused by acceleration of the metabolic rate of the taenia by warming or to restoration of external sources of energy following their depletion. Direct evidence for a link between these changes in metabolism and the fixation of Ca ions by the cell membrane remains to be found. It will be interesting to compare the metabolic effects of epinephrine on the taenia with those on smooth muscles which are excited by catecholamines.

We have mentioned the difficulties involved in drawing conclusions about changes in g_K and g_{Na} from isotope measurements of ionic fluxes (55, 71, *72*, *73*). Recent studies on "depolarized" preparations of taenia coli by Jenkinson & Morton (116) have shown very clearly that norepinephrine causes an increase in K permeability which is blocked by α-adrenergic blocking agents but not by pronethanol. Isoprenaline also causes relaxation of this preparation. Jenkinson & Morton suggest that an increase in g_K may be responsible for the inhibitory action of catecholamines which are mediated through α-receptors.

Excitatory Effects of Epinephrine

The action of epinephrine varies greatly from one preparation to another. Although its dominant effect on gastrointestinal smooth muscle is inhibition, Burnstock (43) has shown that it causes depolarization, the initiation of action potentials, or an increase in their frequency in the muscularis mucosa of pig esophagus. Similar changes in membrane potential probably underly the excitatory effects of epinephrine on the cat nictitating membrane,

on the pelvic viscera of various species (55), on the portal vein (90), and turtle blood vessels (169). The ionic mechanisms involved here may well be similar to those responsible for the excitatory effects of ACh.

The excitatory effect of epinephrine on arterial smooth muscle has been studied by Keatinge (119) who used a modification of the sucrose gap method to record from spiral strips of sheep carotid artery. In these preparations, norepinephrine, epinephrine, and histamine cause an abrupt depolarization of about 10 mV from a resting membrane potential of 60 to 70 mV. In some preparations, this depolarization takes the form of a spike followed by a steady depolarization. Occasionally spikes are superimposed on a steady depolarization. After treatment with inhibitors of oxidative metabolism, norepinephrine causes repetitive firing of plateau-type action potentials, each one of them being associated with an increment in tension.

These effects of epinephrine may be similar to those of large doses of ACh on intestinal muscle in which the depolarization phase of ACh action potentials is maintained for many hundreds of seconds, apparently because of lack of repolarization (59). Metabolic inhibitors may effect passive membrane conductances (71, 72). An increase in g_K would promote repolarization and possibly lead to the repetitive firing of plateau-type action potentials such as those observed by Keatinge (119). Both depolarization and contraction in response to norepinephrine, epinephrine, and histamine are blocked by cooling to 5° C. Low temperature also blocks the contractions associated with depolarization by K-rich solutions, but not contractions in response to intense electrical stimulation.

It is possible that the electrical activity of sheep carotid artery is basically similar to that of turtle arteries, which has been recorded with intracellular electrodes (169). In this preparation, spontaneous action potentials consist of spikes followed by plateaus of 10 to 12 sec duration accompanied by contractions which last for up to 200 seconds. Roddie (169) emphasized that contractions are "associated" with discrete action potentials and not gradual changes in membrane potential. The electrical activity recorded from turtle veins was more variable and ranged from trains of simple spikes with negative after-potentials to "plateau-type" action potentials often showing oscillations similar to those of the guinea pig ureter (14). Epinephrine caused an increase in frequency of the action potentials and summation of contractions in both arteries and veins.

No evidence is available so far which suggests that the ionic basis of the excitatory action of epinephrine differs in any way from that of ACh (43).

Excitatory Action of Acetylcholine, Oxytocin, Tetraethylammonium, and Barium

Acetylcholine causes excitation of most visceral smooth muscles including longitudinal intestinal muscles, bladder, vas deferens, uterus, and ureter. It also causes excitation of multi-unit smooth muscles such as cat

nictitating membrane and rabbit iris (55). The initiation of action potentials in inactive preparations and increase in frequency of firing of action potentials in active preparations are frequently associated with depolarization of the cell membrane (55). More recently, ACh has been shown to have similar actions on the nerve-free chick amnion (69). In one record in this paper, acetylcholine causes the onset of rhythmic slow waves which lead to the intermittent firing of action potentials. Only action potentials trigger contractions. The duration of the plateau-type action potential characteristic of this preparation is decreased by acetylcholine, but this may be an indirect effect caused by the increase in frequency of firing (115).

Low doses of ACh alter the pattern of spontaneous activity in the rat uterus (68, 140). Trains of spikes appear more frequently and each train contains more spikes. Only some cells are depolarized. Others show no change in the resting membrane potential, although a transient depolarization may occur during burst of action potentials. In higher concentrations, ACh causes rapid firing of spikes followed by a maintained depolarization. Contraction is sustained throughout the period of depolarization. This observation is in contrast with the effect of high K on the uterus, which causes a maintained depolarization, but only an initial contraction followed by relaxation. Prolongation of the action potential into a phase of maintained depolarization also occurs in cat longitudinal intestinal muscle where Burnstock & Prosser observed "plateau-type" action potentials of up to 7 sec duration (59). High concentrations of ACh also prolonged the duration of the plateau-type action potentials of the guinea pig ureter. It sometimes causes spontaneous activity in this preparation (114).

In the taenia coli, low doses of ACh increase the rate of depolarization of the prepotentials from which action potentials arise (34). In some cells, prepotentials increase in amplitude. In all cells, prepotentials lead smoothly into action potentials all of which appear to be locally initiated. The amplitude and rate of rise of these action potentials are reduced and the repolarization phase is prolonged and depressed. This latter effect persists after washing out the ACh. During this time, the membrane is no longer depolarized but the action potentials still show a pronounced after-depolarization.

Although the reduction in the amplitude and rate of rise of the action potential by ACh may be secondary effects, because of depolarization of the cell membrane (55), there is little doubt that ACh has a specific effect on the voltage-dependent increase in g_K which brings about the repolarization phase of the action potential. If one of the ionic mechanisms underlying spontaneous activity in smooth muscle is similar to that proposed for cardiac muscle (190), reduction in the level of g_K during repolarization might tend to increase the frequency of firing and to cause an overall depolarization.

One of the difficulties in interpreting the action of a drug on the membrane potential of smooth muscles such as the taenia coli arises from the complex nature of its spontaneous activity, some cells showing evidence of

their pacemaking capacity, whereas others appear to be driven by electrotonic potentials caused by activity in neighbouring cells. High doses of ACh convert the activity of most cells to that of the pacemaker type. When ACh is given in lower doses, however, cells may be encountered with a microelectrode, which show an increase in frequency of firing without any significant depolarization (34). It is difficult to determine if ACh has a differential effect on the local potentials underlying the spontaneous activity of the taenia coli compared with its effect on the action potential mechanism. However, the possibility remains that ACh may increase the excitability of the smooth muscle membrane so that action potentials are triggered at higher membrane potentials. In preparations in which local potentials (slow waves) can be clearly distinguished from action potentials, ACh appears to depress their amplitude (22). This may be a result of the overall depolarization of the cell membrane. ACh may initiate slow waves in quiescent preparations of small intestine (22).

The action of oxytocin on the estrogen uterus resembles that of ACh. Low concentrations cause an increase in the number and frequency of action potentials in a train and the amplitude of the spontaneous contractions. Large doses cause the rapid firing of action potentials leading to a sustained depolarization. Under these conditions, the muscle undergoes a contraction similar to that in response to large doses of ACh (140).

At the skeletal neuromuscular junction, ACh causes an increase in $g_{Na}°$ and $g_K°$, but has little effect on $g_{Cl}°$ (187). It has been postulated by Burnstock (41) that a similar increase in $g_{Na}°$ and $g_K°$ can account for many of its actions in smooth muscle. For example, the action of ACh is abolished in low K or K-free solution, and also by excess K (34), as is the action of oxytocin on the uterus (141). The effect of increasing K concentrations from zero up to isotonic levels is to increase $g_K°$ (176). When ACh acts in low K, the tendency for depolarization caused by an increase in g_{Na} may be counteracted by a tendency to hyperpolarization, resulting from an increase in $g_K°$. In very high K, the already high value of $g_K°$ may counteract any tendency to depolarize, because of an increase in $g_{Na}°$ (the "driving-force" $E_{Na}—E_{EMP}$ being reduced also). The degree of depolarization caused by ACh, in normal solution, depends on the level of RMP. Cells with RMP's of around 35 mV do not undergo further depolarization ($g_{Na}°$ is probably already high). If the RMP is reduced to this level by high K, ACh can still cause depolarization (E_K but not $g_{Na}°$ is reduced).

ACh has no effect in the presence of low Ca, probably because both $g_{Na}°$ and $g_K°$ are already high. Excess Ca potentiates its action. Excess Na also potentiates, as might be expected if ACh increases $g_{Na}°$. The effect of low Na, however, depends on the nature of the Na substitute. Li prevents action potentials from triggering the contractions (6), but does not block the depolarizing action of ACh. Total replacement of Na by Tris blocks the increase in frequency of firing of action potentials but does not block depolarization. If Cl ions are replaced with ethanesulphonate, ACh

still causes depolarization. Bursts of spikes occur, but there is little or no increase in tension.

In summary then, the excitatory action of ACh is probably caused by an increase in g_{Na}^{o} and g_{K}^{o} which may lead to depolarization of the cell membrane. g_{Cl}^{o} may also be increased. ACh also effects the changes in conductance which lead to the initiation of action potentials in smooth muscle, though the details of this mechanism have yet to be clarified. ACh depresses the increase in g_K which occurs during the repolarization phase of the action potential.

The effect of ACh on the rat portal vein is complex (90). Although the membrane is hyperpolarized, action potentials are initiated and these are associated with contractions. It seems possible that ACh may be acting at more than one site in this preparation.

ACh, in concentrations greater than 1 μg/ml, also causes an increase in the frequency of firing of action potentials, and the summation of contractions of turtle vascular smooth muscle (169). The decrease in duration of the plateau-type action potentials of this smooth muscle may have been caused by the increase in their frequency. ACh may also cause contraction of the turtle vein. In low concentrations, however, a decrease in spontaneous activity was observed in both arteries and veins.

Tetraethylammonium (TEA) increases the frequency of the spike discharge in the taenia coli (184, 185). In high concentrations (< 15 mM), an initial increase followed by a decrease in frequency is observed. Prolonged soaking causes a fall in RMP and a decrease in the magnitude of the action potential. TEA causes prolongation of the action potential in the taenia coli as reported for other tissues (185). Action potentials resemble those of cardiac muscle. Prolongation of the repolarization phase may bring the duration of the action potential up to one second. Like ACh, TEA appears to block the increase in g_K which is associated with rapid repolarization.

Very low concentrations of Ba^{++} (threshold 0.05 mM) also cause an increase in the spike frequency of the taenia coli. Higher concentrations (1.4 mM) prolong the duration of action potential by depressing the repolarization phase (185). The RMP is unaffected by these concentrations of Ba^{++}. Higher levels (2.2 mM) cause prolonged depolarization and contraction. These changes are irreversible. If the concentration of Ba^{++} is gradually increased by the addition of small increments at intervals of five minutes, the final effect of 2.6 mM is similar to that of 1.4 mM Ba^{++}, action appears to resemble that of TEA in depressing the repolarization phase of the action potential. Whether its only action is to decrease g_K remains to be seen.

Metabolic Inhibitors

In the taenia coli, a variety of metabolic inhibitors [dinitrophenol (DNP), iodoacetate (IAA), and azide] stimulate at first and then depress mechani-

cal activity (42). The initial effect is associated with depolarization of the membrane and the later depression associated with hyperpolarization.

Casteels (63) found that ouabain also depolarizes the cell membrane of the taenia coli. During this depolarization, spike frequency increases at first, but when the membrane potential reaches 30 to 35 mV all spikes are blocked. Tension development did not follow the increase in spike frequency. This effect of ouabain was antagonized by low Na and high Ca solutions. Casteels suggests that the depolarization might be due to an increase in g_{Na}° or to an inhibition of active cation transport. Restoration of normal solution caused immediate hyperpolarization.

DNP, in concentrations of up to 3×10^{-4} *M*, reduces the frequency of slow waves which are a dominant feature of records from cat and rabbit intestinal muscle (22). Higher concentrations cause depolarization and complete inhibition of slow waves, spikes, and mechanical activity. During complete inhibition by DNP, acetylcholine still produces a rapid, sustained depolarization on which spikes and slow waves are superimposed. The frequency of the slow waves is higher than normal and spikes arise independently off them. Spikes still trigger contractions. High concentrations of ACh cause a sustained depolarization with spikes but no slow waves. Epinephrine still causes hyperpolarization in the presence of DNP. Slow waves appear but these no longer trigger spikes or contractions (22).

The effect of DNP and IAA on pregnant rat uteri has been studied by Marshall & Miller (142) who noted differences in the effect of these drugs on estrogen-dominated uteri (20th to 22nd day of pregnancy) and progesterone-dominated uteri (6th to 9th day). Progesterone uteri show higher resting potentials, smaller spontaneous contractions, reduced electrical excitability, and poorly conducted action potentials compared with uteri under estrogen domination, and they are more sensitive to DNP than estrogen uteri. Marshall & Miller found that 10^{-5} *M* DNP was sufficient to reduce spontaneous or electrically driven contractions as well as those in response to oxytocin. Higher concentrations (10^{-3} *M*) depolarized the cell membrane to a level of −34 mV and abolished all contractions. The only observable effect of 10^{-5} DNP on estrogen uteri was a small depolarization. At 10^{-3} *M*, responses to oxytocin and electrical stimulation were reduced together with spontaneous contractions. The RMP fell to −22 mV, i.e., by about the same amount as in progesterone uteri.

IAA, at a concentration of 2×10^{-4} *M*, abolished spontaneous activity and that in response to electrical stimulation and oxytocin in both groups of muscles without causing any change in membrane potential. If oxytocin was given immediately after spontaneous activity was blocked by IAA, spikes were recorded but no contractions.

These experiments indicate once again that cell metabolism may be more closely linked to membrane phenomena in smooth muscle than in other excitable tissues. Daniel (72) found that DNP caused a net intracellular gain of Na and loss of K which could be accounted for if DNP blocked

the Na pump in smooth muscle as in squid axon. Marshall & Miller, however, doubt whether the large depolarization they observed in both types of uteri could be accounted for by such a change in ionic distribution.

It is tempting to link the depolarization caused by metabolic inhibitors with the inhibition of an electrogenic Na pump (71, 72). There are a number of other instances where inhibition of metabolism leads to depolarization and an increase in metabolism to hyperpolarization (29). However, inhibition of a Na pump cannot account for the blocking action of DNP on excitatory drugs such as ACh and oxytocin. Both DNP and IAA may also effect resting ionic conductances (71). An increase in g_K° would tend to stabilize the cell membrane and block the action of depolarizing drugs. If IAA were more potent than DNP in increasing g_K°, this effect might be sufficient to counteract a tendency to depolarization as a result of metabolic inhibition (29, 31, 32).

Estrogens stimulate metabolic reactions leading to an increase in the production of high energy phosphates (142). Marshall & Miller suggest that this may be the reason why higher concentrations of DNP are needed to depress the activity of uteri in late pregnancy compared with those in early pregnancy. It is difficult to reconcile this interpretation with the ideas put forward by Bueding & Bülbring (29) who suggest that an increase in high energy phosphates cause an increase in Ca binding and stabilization of the cell membrane. This would appear to be characteristic of the progesterone uterus in early pregnancy rather than that under estrogen control (68). It would seem unlikely that the link between metabolism and membrane phenomena should be basically different, in these smooth muscles. Clearly, more work remains to be done before the nature of this link can be established.

On the basis of changes in the response to ACh and epinephrine where the ionic environment of smooth muscle is varied, several theories have been put forward explaining the action of these neurohormones in terms of g_{Na} and g_K. It is now possible to measure membrane conductance directly. By the time this review is published, we hope that many of the speculations described in this section will have already been tested [see Trautwein (189) for discussion of a similar approach to cardiac muscle].

There are at least two directions in which experiments of this kind may well be extended. Little attention has been paid so far to dose-response relationships at the membrane level. Further information along these lines would be helpful in testing current theories of drug-receptor interaction [see Paton (161)]. Finally, it is important to remember that the addition of neurohormones to the isolated organ bath may have different effects on the smooth muscle membrane from those observed when it is liberated in a much higher concentration from nerve terminals or other local stores. The iontophoretic application of drugs to smooth muscle should help to solve this problem.

III. EFFECT OF DRUGS ON AUTONOMIC NERVE-SMOOTH MUSCLE TRANSMISSION

The electrophysiology of transmission from autonomic nerves to smooth muscle was first examined in the guinea pig vas deferens by Burnstock & Holman (48) by means of microelectrode recording from single smooth muscle cells during stimulation of the hypogastric nerves. Since then there have been a number of studies of transmission at various autonomic junctions (11, 12, 13, 44, 45, 49, 50, 51, 53, 54, 92, 93, 127–129, 156–159, 181, 195). The mechanism is essentially similar to that described for other junctions (78, 147). At excitatory junctions, graded potentials (EJP's) appear in response to nerve stimulation; successive EJP's in response to repetitive stimulation may show marked facilitation; when the membrane is depolarized to a critical level because of facilitation, summation, or both, an action potential is initiated and contraction occurs. In some preparations, spontaneous junction potentials (SEJP's) have also been observed (49, 50, 159), which probably represent the spontaneous release of packets of transmitter from the nerve terminals. At inhibitory junctions, nerve stimulation leads to hyperpolarization of the membrane, reduction or cessation of spike activity, and relaxation.

Observations of the changes in membrane potential recorded in smooth muscles in response to transmitter released spontaneously or following nerve stimulation should make it possible to examine the effects of drugs more precisely on pre- and postjunctional sites.

Effect of Drugs on Transmission of Excitation from Sympathetic, Adrenergic Nerves to Smooth Muscle Cells

The action of a number of drugs on transmission of excitation to cells of the guinea pig vas deferens have been studied during stimulation of either the hypogastric nerves or of their postganglionic extensions in the wall of the vas deferens.

Atropine had no detectable effect on the amplitude, frequency, or time course of SEJP's or EJP's in response to stimulation of the hypogastric nerve or to stimulation of intramural nerve fibres (53).

High concentrations (10^{-4} to 5×10^{-4} g/ml) of α-receptor adrenergic blocking agents such as yohimbine, phentolamine, ergotamine, phenoxybenzamine, piperoxan, and tolazoline were needed to block the EJP's in response to nerve stimulation (53, 127). Observation of SEJP's in the presence of high concentrations of α- and β-receptor blocking agents was often difficult because the smooth muscle became spontaneously active under these conditions. However, in all cases, the rate of spontaneous discharge was reduced but not necessarily abolished. During the onset and recovery from yohimbine blockade, the EJP's showed a marked "fatigue" effect, i.e., the first two or three EJP's showed facilitation, but the amplitude of the EJP's which followed was rapidly reduced to zero. This resembles

the effect of yohimbine on amphibian nerve (178), and therefore suggests that its blocking action may be partly due to prejunctional anaesthetic action.

Bretylium initially reduced both EJP's and SEJP's. However, after 30 min exposure, the spontaneous discharge of SEJP's increased in frequency, although the response to nerve stimulation was abolished (53). This result supports the view that bretylium blocks transmission at a prejunctional site. It also emphasizes the need to consider more than one prejunctional site for drug action, since the spontaneous release of norepinephrine, as reflected by the discharge of SEJP's, seems to be affected independently.

Guanethidine, like bretylium, blocked the EJP's, but not the SEJP's. After block of the nerve mediated response, it was possible to stimulate the muscle cells directly (53).

Block of EJP's by procaine was characteristically rapid. SEJP's occurred after block, but after prolonged exposure appeared to be reduced in frequency. There was no sign of the "fatigue" effect on successive EJP's which occurred in the presence of yohimbine (53).

Kuriyama (127) found that various ganglion blockers such as hexamethonium (10^{-4} g/ml) did not alter the electrical responses to transmural stimulation of the vas deferens, although it did block the responses to stimulation of the hypogastric trunk, thus confirming the location of ganglia near the vas deferens (15, 18, 87, 154, 179).

The action of nicotine on transmission from postganglionic sympathetic fibres to the vas deferens has recently been examined in our laboratory (60). The discharge of SEJP's was shown to increase in the presence of nicotine, thus supporting the view that nicotine can act by releasing norepinephrine from postganglionic portions of sympathetic nerves (38, 96).

Records from muscle cells of norepinephrine-depleted vas deferens taken from reserpinized guinea pigs showed that the EJP's were smaller and facilitation slower, so that many stimulating pulses were required before a spike was initiated and contraction occurred (51). Both the frequency and amplitude of the SEJP's were reduced. However, since it was difficult to obtain stable impalements in reserpinized preparations, this effect may be partly explained in terms of its postjunctional action.

The transmission of excitation from sympathetic nerves to smooth muscle cells of the guinea pig mesenteric artery has been studied *in vivo* (181). The mechanism of transmission appears to be similar to the vas deferens, apart from the long delay of not less than 145 msec between stimulation of the nerves and the onset of the EJP compared with a minimum of 6 msec to the vas deferens (127). No studies of drug action have been described yet at this junction. However, it was noted that the depth of urethane-chloralose anaesthesia determined the frequency of stimulation of the sympathetic nerves required to produce facilitation of EJP's sufficient to lead to spike initiation and contraction.

The transmission of excitation from sympathetic nerves to smooth

muscle cells of the retractor penis of anaesthetized dogs has been reported (156–159). Again the pattern of facilitation of EJP's leading to spikes and contraction was observed. In addition, the interesting observation was made that the latency and time course of the EJP was slower in cells in the distal region than in the proximal region of the retractor muscle. This suggests a differential density of innervation in different regions of the same system.

Injection of epinephrine into the animal potentiated the transmission process, i.e., stimulation to the sympathetic nerves which produced only EJP's in the control, produced EJP's which initiated spike activity after the epinephrine injection (157). In contrast, the response to sympathetic nerve stimulation in cells of dogs adrenalectomized seven to nine days previously was reduced, i.e., repetitive stimulation was required before an action potential was initiated. These results were explained in terms of the effect of the treatment on the resting potential; it was lowered after epinephrine injection, and raised in adrenalectomized animals. SEJP's were also recorded in retractor penis cells, especially in the regions where nerves entered the muscle. Adrenalectomy, denervation, and reserpine treatment reduced their frequency (159).

Effect of Drugs on Transmission of Inhibition from Sympathetic, Adrenergic Nerves to the Intestine

Gillespie (92) who worked with the rabbit sympathetic nerve-distal colon preparation could not detect any membrane potential changes in single smooth muscle cells until the nerves were stimulated at frequencies greater than 10 pulses/sec. In stretched preparations, stimulation at high frequencies caused hyperpolarization of the membrane and suppression of both action potentials and slow waves, so that relaxation resulted.

A recent electrophysiological study of transmission of inhibition from perivascular nerves to the taenia coli by Bennett, Burnstock & Holman (12) has confirmed and extended this result. Membrane potential changes were not observed in response to single stimuli in marked contrast to all the known excitatory junctions. The probable reason for this is that the concentration of inhibitory transmitter reaching a muscle cell after a single stimulus is not sufficient. This may be because of a number of factors or combination of factors, including the amount of transmitter released, the number and distance of sources of transmitter release from nerves influencing the muscle cell, and the rate of inactivation of the transmitter. In any case, unlike excitatory junctions, the concentration of transmitter reaching the muscle cells must be increased by repetitive stimulation at frequencies greater than 5 to 10 pulses/sec before there is any detectable change in the membrane potential, after long latencies of up to 270 msec.

The nature and time course of the changes in membrane potential in response to the norepinephrine released on stimulation of perivascular sympa-

thetic nerves are closely paralleled by the effects of directly applied epinephrine (30, 34, 35, 42). The hyperpolarizations produced in the taenia by sympathetic nerve stimulation were abolished by guanethidine and bretylium (12).

Effect of Drugs on Transmission of Inhibition from Intramural Inhibitory Neurons to Intestinal Smooth Muscle

The presence of intramural inhibitory neurons, which are not sympathetic, in Auerbach's plexus with axons supplying the smooth muscle has been established (12, 13, 44, 45, 46, 111).

In the presence of atropine, stimulation of the taenia with single pulses of low duration (0.2 msec) produced transient hyperpolarizations or inhibitory junction potentials (IJP's) of up to 25 mV. Spontaneous hyperpolarizations of the membrane have been seen in some smooth muscle cells (13, 34). They are similar to the IJP's recorded with submaximal stimulation of the nerves, so they may represent "miniature" IJP's or the spontaneous release of transmitter from the intramural inhibitory nerves. However, this will be confirmed only when they can be blocked pharmacologically and shown to have a reversal potential.

The IJP's were blocked by procaine but were unaffected by bretylium and guanethidine. In some experiments with the sucrose gap they were potentiated by atropine or hyoscine and apparently reversed to depolarization by neostigmine or eserine. However, both these last effects were not the result of direct action on the IJP's, but were secondary effects caused by the block or potentiation of excitatory cholinergic nerve fibres which were also being stimulated by the electrodes.

In the presence of norepinephrine, there was a marked decrease in the size of the IJP (10). The explanation is probably that norepinephrine is able to hyperpolarize the membrane primarily by increasing the permeability of the cell membrane to potassium ions. Since the IJP is probably also caused by the movement of potassium ions (11), the amplitude of its response will be small, because the resting potential will already be close to the equilibrium potential for potassium.

Acetylcholine increases the amplitude of the IJP (10). Acetylcholine causes depolarization of the smooth muscle of the taenia probably by increasing the conductance for both potassium and sodium ions (41). Thus, stimulation of the inhibitory nerves would this time lead to a potentiation of the response, because of the larger difference between the resting potential and the potassium equilibrium potential.

In the presence of atropine, nicotine has been shown to hyperpolarize the membrane and thus secondarily reduce the amplitude of the IJP, while nicotine depolarized the membrane and slightly potentiated the IJP in the absence of atropine (60). None of the drugs which have been tested so far have specifically blocked the IJP, and the nature of the transmitter substance remains obscure.

Effect of Drugs on Transmission of Excitation from Parasympathetic, Cholinergic Nerves to Smooth Muscle

EJP's have been recorded in smooth muscle cells of three preparations in response to stimulation of cholinergic nerves, namely the isolated nerve-urinary bladder strip of the rabbit (195) (which may also include adrenergic nerves), the rabbit pelvic nerve-distal colon (92, 94), and the taenia of the guinea pig (10, 45).

The EJP's in these preparations resembled EJP's recorded in adrenergic preparations in many ways. They have been observed in response to a single stimulus, they sum and facilitate in response to repetitive stimulation, although facilitation appears to be less marked than in adrenergic transmission in the guinea pig vas deferens. The latency of the EJP is long [35 to 100 msec in the bladder, about 220 msec in the colon (transmural stimulation), and about 200 msec in the taenia] compared to a minimum latency of 6 msec for sympathetic EJP's in the vas deferens. This variability may be explained in terms of the distance of the muscle from the points of release of transmitter from the nerve. Furthermore, these longer distances may also account for the absence of reports of miniature EJP's in these preparations.

It is interesting to note that unlike the vas deferens, in these three preparations EJP's were not seen in all cells penetrated with the microelectrodes upon stimulation of the cholinergic nerves. In some cells, this may have been due to poor microelectrode penetration, but it could indicate that different cells in the population are "innervated" by different types of nerves. Excitatory nerves may innervate groups of cells only, since the rest of the cell population can be excited by intermuscle fibre spread of excitation.

The action of drugs on cholinergic excitatory EJP's in the bladder and colon has not yet been reported. However, the EJP's recorded in the guinea pig taenia with the sucrose gap technique were abolished by atropine and potentiated by neostigmine (45).

Effect of Drugs on Transmission of Inhibition from Parasympathetic, Cholinergic Nerves to Smooth Muscle

Hyperpolarizations have been recorded in single smooth muscle cells of the anaesthetized dog retractor penis during stimulation of the parasympathetic nerves (156, 158). Stimulation of pre- or postganglionic parasympathetic nerves with single pulses did not produce any membrane potential changes. However, with repetitive stimulation of not less than 1 to 3 pulse/sec hyperpolarization of up to 17 mV was produced. The shortest latent period for the response was about 500 msec at higher frequencies of stimulation, but became longer again above 50 pulses/sec.

Hyperpolarizations were also produced by direct application of acetylcholine, and the nerve-mediated hyperpolarizations were blocked by atro-

pine and *d*-tubocurarine. Eserine potentiated the response in the sense that it reduced the latency and that lower frequencies of stimulation than usual were capable of producing a response.

IV. CONCLUSIONS

We hope that this account of the work carried out so far on the action of drugs on the smooth muscle membrane and on autonomic transmission will encourage more experiments of the same kind. When more is known of the sites and specific mechanisms of drug action at various neuroeffector junctions, it may be possible to interpret the actions of drugs on preparations containing more than one type of junction. When this has been done on *in vitro* preparations, then it should be possible to go further towards the interpretation of the *in vivo* action of drugs. It is hoped that the circular arguments so common in the current literature in this field will disappear and that we will no longer be expected to read innumerable speculative papers about the effect of drugs of unknown specificity on tissues with unknown sites of action.

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