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A Survey of the New Findings Presented and the Discussions Arising During Sessions I, II and III

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A survey of the new findings presented and the discussions arising during sessions I, II and III†

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One of the earliest studies on the physiology of smooth muscle was that reported by Engelmann over 100 years ago. In setting the stage for this discussion on new developments in smooth muscle physiology, Professor Bozler recalled Engelmann's description of the ureter as a 'giant hollow muscle fibre'. Recent work on the passive electrical properties of smooth muscle has shown that Engelmann's concept of the syncytial behaviour of smooth muscle is true for a great many smooth muscles – perhaps for all vertebrate smooth muscles. When smooth muscle cells come into contact they interact with each other so as to form a tissue. In this sense, a community of smooth muscle cells is analogous with the liver, epithelial tissues and the heart. One can contrast this 'collective' behaviour of smooth muscle cells with the separate identity maintained by most nerve cells and skeletal muscle fibres.

Communication between smooth muscle cells

Dr Bennett showed how it is possible, theoretically, to assemble a bundle of individual smooth muscle cells so that the bundle behaves as if it were a single muscle fibre (Bennett 1972). His analysis confirmed and extended the previous model of Tomita (1966) and the predictions of Engelmann and Bozler (1948). The electrical characteristics of the smooth muscle membrane that emerge from Dr Bennett's model are of interest. Membrane capacity (capacitance of unit area of membrane) is about 0.8 $\mu F/cm^2$ and membrane resistance (the resistance of unit area of the cell membrane) is of the order of 100 $k\Omega\ cm^2$. The value of the time constant of a bundle of smooth muscle cells (as measured from the change in membrane potential caused by a square pulse of current applied to the whole bundle) is about 100 ms (see Tomita 1970). The value of membrane capacitance obtained by Tomita and others from the 'foot' of a propagating action potential is 1.5 to 3 $\mu F/cm^2$. Membrane resistance according to Tomita (1970) would be up to 60 $k\Omega\ cm^2$. Although there is some disparity between these values and those of Dr Bennett's, it seems very likely that the conductance of smooth muscle membrane is quite low – even by comparison with the slow skeletal muscle fibres of amphibia, whereas membrane capacitance may be similar to that of other excitable cells (about 1 $\mu F/cm^2$).

We know very little about the electrical properties of the junctions between smooth muscle cells. In the taenia coli, the impedance to flow of longitudinal current is two to three times greater than would be expected if there was no resistance to the flow of current between neighbouring cells and longitudinal resistance was determined only by the specific resistivity of the smooth muscle myoplasm. Tomita (1970) has summarized evidence suggesting that the junctions between cells have a significant capacitance but he pointed out that it is impossible to relate any estimate of junctional resistance and capacitance to unit area of

† This survey is based on material presented during the meeting. Manuscripts of these papers were not available to the author.

cell membrane since we have no idea of the area of membrane involved in intercellular communication.

During the last few years, studies have been carried out on many different smooth muscles, using the method of polarizing bundles or strips of muscle to investigate their passive electrical properties. It is of interest that so far, with the exception of the very thin longitudinal layer of the mouse vas deferens, spread of electrotonus has always been shown to occur (see, for example, Kuriyama, Osa & Tasaki 1970; Creed 1971; Mekata 1971). Perhaps the degree of electrical communication between cells may not be the only determinant of the 'multi' versus 'single' unit behaviour of different smooth muscles, as defined by Professor Bozler. The ability or otherwise of different smooth muscles to generate action potentials might also be important.

A key question in smooth muscle research (and in the general context of interaction between cells) is the morphology of regions of high permeability between the interiors of neighbouring cells. Close apposition of the membranes of neighbouring cells has been observed to occur in many different smooth muscles and Dr Gabella showed some elegant electronmicrographs of these structures. They appear to differ from the tight junctions between epithelial cells (where the outer leaflets of neighbouring membranes seem to fuse) since colloidal lanthanum can penetrate between closely apposed membranes of smooth muscle cells but not through tight junctions. Lanthanum also penetrates the small (2 nm) gap junctions (Revel, Olsen & Karnovsky 1967) which are thought to be sites of communication between certain types of cells (Brightman & Reese 1969); it is of interest that gap junctions have also been found in some smooth muscles (Uehara & Burnstock 1969). Dr Gabella discussed the importance of the methods used in preparing tissues for electronmicroscopy, in determining the appearance of regions of close contact between cells. He felt that at present all such regions might be regarded as candidates for electrical interaction. He pointed out that regions of close apposition of the type referred to as 'nexuses' by Dewey & Barr (1962) have yet to be seen in the longitudinal layer of the small intestine where there is good physiological evidence for syncytial behaviour. However, it must be realized that the distance separating the membranes of neighbouring cells does not, in itself, give any information about permeability of these membranes. Dr Gabella drew our attention to other types of specialized contact between the membranes of neighbouring smooth muscle cells. A fairly common finding is the intrusion of a bulbous process from one cell into one of its neighbours. When this occurs, the separation between the neighbouring cell membranes is such that no basement membrane material may be present in the gap (see also Burnstock 1970).

One is left with the impression that smooth muscle cells are capable of forming a variety of structures when their membranes come into close contact with each other but it is still not clear which, if any of these regions of contact are sites of high permeability.

Regulation of intracellular ions

Apart from the question of intercellular communication, papers on the morphology of smooth muscle presented by Dr Gabella and Dr Devine drew attention to other features of the smooth muscle cell which may be of significance in relation to one of the principal problems that concerned this meeting. This is the question of the regulation of the concentration of ionized calcium (Ca^{2+}) in the cytoplasm, that is available to the smooth muscle troponin system to activate contraction. Such structures might include the following:

(1) The caveoli which abound in rows along the surface of smooth muscle cells.

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(2) The 'rough' and 'smooth' endoplasmic reticulum. (Both Dr Gabella and Dr Devine emphasized the close proximity of the endoplasmic reticulum with the caveoli and surface membrane of the smooth muscle cell.)

(3) Mitochondria.

(4) The smooth muscle cell membrane itself with its characteristic covering of basement membrane material.

These morphological papers provided a provocative background for physiological studies on the way in which smooth muscle cells might deal with their ions. It is possible, for instance, that the cell membrane may be provided with a micro-environment that depends on the properties of its surrounding basement membrane. If this covering contains proteoglycan molecules similar to those of connective tissues (hyaluronic acid and/or chondroitin sulphate protein complexes) the smooth muscle membrane is likely to be surrounded by a barrier to the permeation of large molecules and perhaps, more importantly, by a large number of fixed negative charges.

Dr Brading discussed the difficulties involved in estimating values for the permeability of the membrane for Na^+, K^+ and Cl^- ions (P_{Na}, P_K and P_{Cl}). The complex shape of efflux curves determined by tracer experiments under steady-state conditions is well known. Potassium presents somewhat less of a problem than Na^+, since the instantaneous rate constants for its efflux reach a constant value after about 5 min and it seems reasonable to assume that this represents membrane limited exchange. However, the amount of tissue K^+ that has to be fitted into the extracellular space is so large that it is necessary to assume that some K^+ is concentrated in an extracellular compartment, perhaps loosely bound to negative charges on or near the cell membrane. The exchange of Na^+ is more complex. Again, the fastest components are much too large for the extracellular space. But the instantaneous rate constants for Na^+ exchange never reach a steady level (Brading 1971). Attempts to estimate P_{Na} from studies on Na fluxes must depend upon a number of assumptions including, for example, acceptance of the hypothesis that the resting membrane potential and membrane resistance can be explained by the constant field equation. Dr Brading reminded us that other models are available which could explain the ionic phenomena observed in smooth muscle (see Jones 1970). She described some of her recent work on tissues in a steady state, but under conditions of high intracellular Na^+ or K^+. Potassium efflux from preparations loaded with K^+ (by exposure to Na^+-free solutions) showed simple kinetics, but Na^+ efflux from Na^+ loaded preparations (K^+-free solution) was characterized by a continually decreasing value for the instantaneous rate constant. This was so in spite of the fact that in these latter experiments the activity of the Na^+–K^+ pump must have been very low. Dr Brading also studied the loss of K^+ and Na^+ from loaded tissue into sucrose solutions. Both ions were lost from the tissue rapidly. Loss of K^+ was unaffected by the presence or otherwise of Ca^{2+} but loss of Na^+ was increased in the presence of Ca^{2+}, suggesting a possible exchange between Na^+ and Ca^{2+}.

Because the surface area to volume ratio is large in smooth muscles a significant number of cations might be bound to negatively charged sites associated with the cell membrane or its basement membrane. If the properties of these binding sites can be determined then it may be possible to explain the efflux curves for Na^+ and K^+ and to be more certain about which component represents membrane limited exchange. At present, many uncertainties are involved in attempts to estimate P_{Na} and P_K from studies using radioisotopes.

Professor Casteels described some of the properties of the Na^+–K^+ pump in smooth muscle.

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He reviewed the evidence that this pump can make a substantial contribution to the membrane potential if it is activated by an increase in intracellular Na^+ and if membrane conductance is reduced by replacing Cl^- with an impermeable anion. Professor Casteels discussed the possible contribution of an electrogenic Na^+–K^+ pump to the resting membrane potential of the taenia coli. It may be argued that an increase in the activity of the pump could cause a change in membrane potential as a consequence of the depletion of K^+ ions from the 'micro-environment' of the smooth muscle cell. It seems unlikely that this could explain the hyperpolarization observed when Na^+ loaded muscles are returned to K^+ solutions. It has been shown (Casteels 1971) that P_K is higher in the presence of Cl^- ions than in the presence of less permeable anions. Yet the hyperpolarization observed when Na^+ loaded muscles are exposed to K^+ solutions is much greater if Cl^- is replaced by a less permeable anion.

In attempting to explain the ionic basis of the resting membrane potential of smooth muscle yet another problem arises. This is the need to evoke not only a pump for cations but also an anion pump to explain the distribution and permeability of Cl^-. The way in which smooth muscle cells deal with their anions is a question which has yet to be resolved.

Control of intracellular Ca^{2+} *ions*

Dr van Breemen described the way in which lanthanum (La^{3+}) can be used to displace Ca^{2+} from extracellular binding sites in order to simplify analysis of the control of intracellular Ca^{2+}. He presented evidence that the contractures induced by high K^+ and by depletion of $[Na^+]_o$ were associated with an influx of Ca^{2+}, whereas contraction of smooth muscles by noradrenaline and angiotensin was due mainly to the release of Ca^{2+} bound inside the cell membrane. Since it is unlikely that angiotensin can penetrate smooth muscle cells it would seem that the action of this agonist may be due to interaction at the outer cell membrane which leads to the release of Ca^{2+} from the inner surface of the membrane. Dr van Breemen's studies suggest that the Ca^{2+} ions bound to the inside of the membrane are normally replaced by extracellular Ca^{2+} since only a single response to noradrenaline could be observed in the presence of La^{3+}. If drugs such as verapamil were used to block stimulated Ca^{2+} influx but not extracellular Ca^{2+} binding, noradrenaline contractions could be repeated. A further important point made by Dr van Breemen was that La^{3+} treatment could result in relaxation, in spite of the fact that Ca^{2+} efflux was blocked. This suggests that relaxation can be brought about by binding of Ca^{2+} by intracellular components such as mitochondria or endoplasmic reticulum.

Although contractions under physiological conditions may be partly or largely associated with the release of intracellularly bound Ca^{2+}, and in spite of the fact that membrane permeability for Ca^{2+} is low, some mechanism must exist to maintain a steady state for the total intracellular calcium content. This point was also raised by Professor Tomita who felt that, since there was good evidence that the action potentials of smooth muscles were associated with an inward current carried by Ca^{2+} ions, some process must occur to prevent an accumulation of cell calcium. Dr van Breemen presented evidence that ATP might be involved in Ca^{2+} efflux though he had no suggestion to offer as to the details of this process.

Professor Reuter's paper was also concerned with the question of the control of intracellular Ca^{2+}. He provided evidence that contractures induced by differing stimuli (high extracellular K^+ or low Na^+) may increase intracellular Ca^{2+} by different mechanisms. It is well known that the magnitude of K^+ contractures is related to changes in cell membrane potential. Contractures induced by low Na^+ cannot be so explained. Reuter found that the tension developed in

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low Na^+ solutions was constant, if the ratio of $[Ca^{2+}]:[Na^+]^2$ was kept constant; his results indicate competition between these ions for some step in the process activating contraction. Both Reuter and van Breemen found a net uptake of Ca^{2+} in low Na^+ solutions. Reuter found that this uptake was greatest when Na^+ was replaced by sucrose and least when Li^+ replaced Na^+.

A passive efflux of Ca^{2+}, coupled to a passive influx of Na^+ has been found to occur in a number of excitable cells (see, for example, Reuter & Seitz 1968; Baker, Blaustein, Hodgkin & Steinhardt 1969) and Reuter gave evidence that this might also be a characteristic of smooth muscle membrane. He considered the cause of contractures in low Na^+ to be as follows: 'In the absence of $[Na^+]_o$ the transport sites at the outer surface of the membrane should be mainly occupied by Ca^{2+}. Since in this condition there is no inwardly directed Na^+ gradient, net outward transport of Ca^{2+} should be inhibited while net inward movement of Ca^{2+} increases.'

Many of the results described by Reuter and Dr van Breemen were complementary, and they make an important contribution to our understanding of Ca^{2+} metabolism in smooth muscle which cannot be adequately summarized here. They were in agreement in the interpretation of much of their data. However, Reuter felt that an important, but not the only mechanism for the maintenance of a steady-state value for cell Ca^{2+} was a passive efflux of intracellular Ca^{2+} in exchange for a passive influx of Na^+, whereas Dr van Breemen felt that his evidence suggested that this was unlikely to be the key mechanism.

Professor Tomita had also studied contractures in Na^+ free solutions. He found that K^+ contractures of the taenia coli were maintained if the K^+ solution was replaced by sucrose. When quite small amounts of Na^+ (greater than 5 mmol/l) were added to the sucrose, the muscle relaxed. This finding could be taken to indicate that the exchange of cell Ca^{2+} for extracellular Na^+ can bring about a decrease in cell Ca^{2+}. However, agents which blocked the influx of Ca^{2+} (including Mg^{2+}, La^{3+} etc.) also caused relaxation if they were added to the sucrose solution. This suggests that during the sucrose contracture there was a continuing influx of Ca^{2+} through the cell membrane. If Ca^{2+} and Na^+ ions competed for transport sites in the cell membrane, then an alternative explanation can be given for the relaxing effect of Na^+, i.e. a decrease in Ca^{2+} influx. As Reuter had already suggested, both mechanisms may be involved.

It seems clear that there are a number of different mechanisms leading to an increase in $[Ca^{2+}]_{in}$ to activate contraction in smooth muscle. These might include the following:

(1) Depolarization may lead to an increase in P_{Ca} and a net influx of Ca^{2+}. (The action potentials of smooth muscle, for example, are probably due in part to a membrane potential dependent increase in P_{Ca}, and an inward Ca^{2+} current which might contribute to excitation-contraction coupling.)

(2) Depolarization may release Ca^{2+} bound to the inside of the cell membrane.

(3) Depolarization or Ca^{2+} entering the cell during depolarization may release Ca^{2+} from intracellular stores.

(4) Certain drugs may release Ca^{2+} bound inside the cell without necessarily causing a change in membrane potential.

It is necessary to be even more speculative in attempting to summarize the mechanisms which may be involved in sequestering Ca^{2+} within the cell and in maintaining a steady state for total tissue calcium.

(1) Accumulation of Ca^{2+} by mitochondria and endoplasmic reticulum. (There is no doubt

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about the important role of mitochondria in buffering intracellular Ca^{2+} in other cells (Baker 1971). Dr Devine presented evidence for the accumulation of strontium within the endoplasmic reticulum (see Devine, Somlyo & Somlyo 1972), but the precise role of these elements in the regulation of Ca^{2+} remains to be clarified).

(2) Passive exchange of intracellular Ca^{2+} with extracellular Na^{+}.

(3) The outward movement of Ca^{2+} across the cell membrane by an active transport process.

Origin of spontaneous activity

Professor Tomita reviewed the factors which determine the spontaneous electrical activity characteristic of many smooth muscles. The Ca^{2+} dependent action potentials ('spikes') of the gastrointestinal tract of many species can be differentiated from a second component – the slow wave – which is dependent on extracellular Na^{+}. Slow waves set the rate and pattern of the contractile activity of these smooth muscles, but action potentials seem to be the events which trigger contraction. Slow wave frequency is very dependent on temperature, suggesting that their initiation might be closely linked to cell metabolism. Tomita proposed that slow waves might be generated by an increase in P_{Na} caused by membrane depolarization. He thought that cell metabolism might be linked to such a change in permeability by the active extrusion of Ca^{2+} across the cell membrane; as Ca^{2+} is removed from binding sites inside the cell membrane by an active transport process, P_K might fall and the membrane depolarize. This depolarization could cause a membrane potential dependent increase in P_{Na}. The idea that removal of Ca^{2+} bound to the inside of the cell membrane could cause a decrease in P_K, is of considerable interest and some of the implications of this hypothesis were discussed during later sessions of the meeting (see below).

Tomita demonstrated that the taenia coli is able to generate large, all-or-nothing action potentials during prolonged exposure to isotonic $CaCl_2$. Under thesec conditions the inward current during the rising phase must be entirely due to an influx of Ca^{2+}. It is of interest that the taenia eventually undergoes depolarization in isotonic $CaCl_2$ and this is associated with the spontaneous discharge of Ca^{2+} action potentials. Thus Na^{+} ions are not essential for all types of spontaneous activity.

Actions of nerve stimulation and neurotransmitters

Further aspects of the physiology of smooth muscle discussed by the meeting were the ways in which changes in membrane potential could be induced by stimulation of its nerve supply, by neurotransmitters or by agonist drugs.

Dr Bennett has used his model of the electrical properties of bundles, tubes and sheets of smooth muscle cells to predict the time course of the excitatory junction potentials (e.j.p.) evoked by stimulation of motor nerves (Bennett 1972). The e.j.p. recorded from different smooth muscles vary greatly in their duration from about 1 s (gastrointestinal tract) to about 150 ms (mouse vas deferens) (Holman 1970). The rate of rise and initial rate of decay also vary greatly. Depending upon the way in which the smooth muscle cells are arranged in relation to sites of transmitter release the shape of the e.j.p. is dominated by a high local concentration of transmitter or by its diffusion from more distant sites of release. In the mouse vas deferens, transmission is effected mainly through sites of high local concentration where axon varicosities make close contact with smooth muscle cells. In the gastrointestinal tract and in vascular smooth muscle transmission is due to a more widespread increase in the concentration of transmitter.

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Dr Bennett suggested that close contacts between smooth muscle cells and axon varicosities, characteristic of the vas deferens, might be associated with regions of the smooth cell where there was a high density of receptors.

Professor Gillespie discussed some of the properties of the rat anococcygeus, a 'strap' of muscle surrounding the terminal rectum which is unusual since it has a tendon. This smooth muscle also shows unusual responses to nerve stimulation. The anococcygeus has a dense noradrenergic motor innervation; graded stimulation of these nerves causes small depolarizations which suddenly develop into a much larger change in membrane potential. At first sight, intracellular records from this muscle seem to resemble those obtained from the mouse vas deferens (Furness & Burnstock 1969), but the mouse vas deferens shows a much more graded behaviour in response to increasing strengths of stimulation. It will be interesting to know how the anococcygeus responds to direct electrical stimulation. This muscle also has an inhibitory innervation; stimulation of these nerves causes relaxation which was not associated with any change in membrane potential. It therefore seems likely that relaxation of the anococcygeal muscle is due to a different mechanism from that underlying the relaxation produced by the α-inhibitory actions of catecholamines or by the intrinsic inhibitory nerve cells present within the plexuses of the gastrointestinal tract.

Dr Furness reviewed the actions of the intrinsic inhibitory neurons present in the gastrointestinal tract. He gave an account of the arguments for and against the possibility that they may act through the release of ATP which fulfilled many of the criteria which are generally accepted for the identification of neuro-transmitters (Burnstock 1972). It is clear that the ATP hypothesis is a challenging one which should provoke many new experiments on these neurons and the way in which they inhibit smooth muscle. In this case, inhibition seems to be due to a large hyperpolarization which is probably caused by an increase in P_K. Dr Furness stressed the importance of these neurons in reflexes of the gastrointestinal tract including control of the lower oesophageal sphincter, receptive relaxation of the stomach, and the descending inhibition of the intestine during peristalsis. These neurons appear to be important in a 'cascade of inhibitory reflexes aiding the passage of food through the gut'.

Professor Bülbring reminded us of the differing mechanisms underlying the α and β actions of catecholamines. Both receptor types cause inhibition of the taenia coli. She presented an attractive hypothesis to explain these actions which will also challenge us for some time to come. Just as the β-inhibitory action of catecholamines depends on the activation of a membrane enzyme, adenyl cyclase, so their α action might also depend upon the activation of an α membrane enzyme. In contrast to the activation of the β enzyme which leads to Ca^{2+} *uptake* into storage sites, the activation of the α enzyme might lead to the *release of* Ca^{2+} from intracellular storage sites, resulting in an increase in the binding of Ca^{2+} on the inside of the cell membrane and to an increase in the active extrusion of Ca^{2+}. Binding of Ca^{2+} on the inside of the cell membrane may well be an important determinant of the membrane permeability to K^+ (as suggested earlier by Tomita). Cells in which the amount of membrane bound Ca^{2+} is relatively low would be expected to have a relatively low P_K (e.g. the taenia coli). Activation of the postulated membrane enzyme might lead, therefore, to an increase in Ca^{2+} binding and to an increase in P_K, hyperpolarization and inhibition.

In the oestrogen and progesterone dominated guinea-pig uterus there is much more membrane bound Ca^{2+} than in the taenia, and this could explain its relatively high P_K. Moreover, the α action of catecholamines on the uterus is excitatory (see below), indicating

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that, in this tissue, it does not cause a significant increase in Ca^{2+} binding on the inside of the membrane.

Dr Marshall presented very compelling evidence that the β inhibitory action of catecholamines on the oestrogen dominated rat uterus was associated with an increase in the intracellular concentration of cyclic AMP. This finding is in accordance with the widely accepted theory that the β-receptor is coupled to adenyl cyclase. The increase in cyclic AMP was correlated with a reduction of the calcium content of the cells. The hyperpolarization associated with the action of isoprenaline on the rat uterus would seem to be a side effect of the inhibition caused by isoprenaline and not the key to its action in relaxing smooth muscle.

Hyperpolarization, which is associated with an increase in the activity of the Na^+–K^+ pump, occurs after the guinea-pig ileum has been exposed to large doses of acetylcholine. Dr Bolton has shown that depolarization by carbachol and acetylcholine is due to an increase in cation permeability (Bolton 1972). This would be expected to lead to an increase in intracellular Na^+. As demonstrated by Professor Casteels (see above) and others (Taylor, Paton & Daniel 1970) the activity of the smooth muscle pump, like that of many other tissues, is probably geared to the level of intracellular Na^+. Dr Bolton showed that during prolonged exposure to carbachol the action of the pump was sufficient to modify the level of depolarization produced by this agonist. This finding is of interest in relation to the question of the reversal potential for carbachol. Dr Bolton suggested that important aspects of agonist action on smooth muscle were a disturbance of ionic gradients and the 'turning on' of mechanisms which would restore ionic batteries.

One of the compelling reasons for the continuing interest of pharmacologists in smooth muscle is that this tissue presents a large surface area of membrane covered with a great variety of receptors mediating both excitation and inhibition. We know that inhibition may be caused by several different membrane mechanisms. The α and β inhibitory actions of catecholamines, and the action of intrinsic inhibitory nerves of the gastrointestinal tract all appear to be operating in different ways. Dr Szurszewski discussed an example of the excitation of smooth muscle which is due to a different mechanism from the classical increase in cation permeability discussed by Dr Bolton. Excitation of the guinea-pig uterus by noradrenaline can be explained by an increase in permeability for Cl^- ions. This leads to depolarization since the equilibrium potential (E_{Cl})for Cl^- is considerably less than the resting membrane potential. It will be interesting to know if alterations in E_{Cl} can change the action of noradrenaline from excitation to inhibition.

Conclusion

Much new information about the physiology of smooth muscle was presented during this meeting but we were also confronted with many unsolved problems and an impressive series of hypotheses.

Some, but by no means all, of the current problems in smooth muscle research are the following:

(1) The physiology and morphology of communication between cells and the cause of 'multi-unit' versus 'single-unit' behaviour of different smooth muscles.

(2) The mechanisms by which a steady-state level of total tissue calcium is achieved.

(3) The relative importance of the various possible sources of Ca^{2+} for excitation–contraction coupling.

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(4) Identification of the cation which is *mainly* responsible for carrying the inward current during the action potential in different smooth muscles, under normal conditions; and the reason why some smooth muscles do not appear capable of generating action potentials.

(5) The origin of spontaneous activity in different smooth muscles.

(6) The control of membrane potential by neurotransmitters and hormones and the possibility that membrane bound enzymes may be closely linked to receptor sites for these agents.

(7) The non-adrenergic, non-cholinergic inhibition of gastro-intestinal muscles mediated by enteric neurons, and the possibility that ATP is a neurotransmitter.

(8) The long-term effects on smooth muscle of the pattern of its innervation. Do close contacts between terminal axon varicosities and the smooth muscle membrane influence the distribution of receptors for the appropriate neurotransmitter?

Most of the efforts of smooth muscle physiologists in the past have been devoted to a relatively small number of preparations. As a result of this we are probably inclined to generalize too easily and too quickly. It is true that physiologists should not be concerned with the acquisition of data for its own sake, but at the moment we are greatly in need of more data about the behaviour of different smooth muscles. This meeting would seem to have provided enough hypotheses to stimulate smooth muscle research for some time to come.

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