MINIREVIEW

Ionic Conductances in Dissociated Smooth Muscle Cells (43234)

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Smooth muscles constitute the major portion of the walls of hollow organs in the body, such as the gastrointestinal tract, the urinary bladder, and blood vessels. Mammalian smooth muscle is made up of small, mononucleate, spindle-shaped cells 20–400 μm in length and about 5–10 μm in diameter. Neighboring cells may be connected to one another through gap junctions, thus facilitating the spread of excitation from cell to cell. In this way, the electrical activities of adjacent smooth muscle cells are synchronized and coupled to form functional syncytial units. Smooth muscles perform a variety of functions. In the cardiovascular system, they control the diameter of blood vessels important in regulating blood pressure and the vascular sphincters that determine the distribution of blood to different areas within the body. In the gastrointestinal system, they control the movement of food and chyme down the tract. To understand the electrical properties of smooth muscle, the individual ionic conductances responsible for the excitability of its membrane have to be elucidated. Standard smooth muscle preparations are notoriously difficult to study by conventional microelectrode techniques. Cells tend to be small and flat thus making microelectrode impalement without damaging the membranes extremely difficult. Spontaneous mechanical movement may also be present and can further compound this difficulty. The presence of neural and humoral influences capable of generating and modulating electrical responses in intact tissues may further complicate the interpretation of the data. To circumvent the inherent difficulties associated with intact smooth muscle, enzymatically dissociated single smooth muscle cells have been used in electrophysiologic studies. Single cells isolated from the toad (*Bufo marinus*) stomach muscularis have been studied successfully with intracellular microelectrodes to determine the ionic basis of their action potential (1, 2). Walsh and Singer (3) have also demonstrated that these isolated smooth muscle cells can be voltage clamped with two microelectrodes and they have presented evidence for the presence of a Ca^{2+} inward current, a Ca^{2+}-activated K^+ peak outward current and a steady-state K^+ outward current. Within the past few years, the powerful patch-clamp technique (4) has been applied to a variety of dissociated smooth muscle cells using cell-attached patches, excised patches, and whole-cell recording, and has yielded much information and insight into the electrophysiologic characteristics of smooth muscle cells. There are excellent reviews on the patch-clamp technique (4, 5). From single-channel recordings, the amplitude and duration of the unitary current, the selectivity of the channel, the gating properties that control the opening and closing of a channel, and the pharmacology of an ion channel are some of the parameters that can be studied with the technique. The whole-cell variant of the patch-clamp technique allows measurement of macroscopic currents similar to those obtained by traditional microelectrode voltage clamp, but offers the advantage of internal perfusion with the electrode filling solution and the ability to work with very small cells. It is the aim of this review to summarize the ionic currents flowing through individual channels that have been observed in various dissociated single smooth muscle cells as determined by the patch-clamp technique. Several recent reviews are available on the electrophysiology of gastrointestinal smooth muscle cells (6–8) and vascular smooth muscle cells (9). This review is not intended to be exhaustive but will concentrate specifically on some of the similarities and differences of the ionic conductances found in

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dispersed gastrointestinal and vascular smooth muscle cells.

Cell Dissociation Techniques

Studies of single ion channels in smooth muscle membrane depend on dispersing smooth muscle tissue into single smooth muscle cells. Methods currently in use to obtain single cells derive from the pioneering work of Bagby *et al.* (10). They used solutions with collagenase and trypsin to isolate living, contracting smooth muscle cells from the stomach of adult *Bufo marinus*. Fay *et al.* (11) have continued to refine the procedures for isolating single stomach smooth muscle cells. A recent review is available describing the properties of isolated smooth muscle cells from the gut (12). Similar techniques are used to isolate other gastrointestinal as well as an assorted number of vascular smooth muscle cells.

Although single-dispersed cells can provide direct information about the ionic channels that are present in their membranes as well as their modulation, it must be remembered that because of the enzymatic treatments, the normal physiologic surrounding of the channels may be perturbed to the extent that electrical events routinely observed from intact smooth muscle tissue are sometimes not seen in isolated single cells.

Macroscopic Currents and Ion Channels

Whole-cell electrophysiologic studies on single smooth muscle cells revealed the existence of four major macroscopic currents: (i) an inward Ca^{2+} current that accounts for the depolarization phase of the action potential; (ii) an outward Ca^{2+}-activated K^+ current that peaks and then decline to a steady-state current; (iii) a transient early outward current; and (iv) a steady-state outward current (13–15). In addition, currents passing through other types of channel have also been observed.

The following are surveys on the properties of some of the major ion channels that have been observed from dissociated gastrointestinal and vascular smooth muscle cells.

Calcium Channels. Calcium ions are responsible for initiating the mechanical response in smooth muscle cells. They may be mobilized from internal calcium stores within the cell as well as from the extracellular fluid. There are two major routes by which Ca^{2+} ions from the external solution may cross the cell membrane and thereby influence the contractile apparatus. One pathway that has been proposed is through voltage-gated calcium channels and the other is through receptor-operated calcium channels, the functions of which are regulated, at least partially, by agonist-receptor interactions. Any single smooth muscle cell may, however, contain several different types of voltage-activated Ca^{2+} channels. Since voltage-activated Ca^{2+} channels are also activated by norepinephrine, a recent review (9) has proposed an interesting hypothesis that in some vascular smooth muscle cells, receptor-operated channels and voltage-gated Ca^{2+} channels are in fact one and the same. Further single-channel studies will be needed to determine whether this hypothesis is indeed valid and whether it is generally applicable to different types of smooth muscle cells.

Inward currents in smooth muscle cells were suggested to be carried by Ca^{2+} since they were not inhibited by tetrodotoxin (TTX), a specific blocker of voltage-gated Na^+ channels, but abolished by the calcium antagonist, nifedipine. Calcium currents activated by membrane depolarization have been identified and described in a variety of dissociated gastrointestinal smooth muscle cells, such as guinea pig and rabbit stomach (16), amphibian stomach (17), toad stomach (18), guinea pig taenia coli (19–22), and rabbit longitudinal colonic muscle (23, 24). An interesting observation was that action potentials could not be elicited from some of the dispersed cat and rabbit colon cells (24) in response to depolarizing current pulses, prompting the authors to suggest that some of the circular muscle fibers may contract by an action-potential independent mechanism. However, it is also possible that the absence of action potentials is an artifact resulting from the enzymatic treatments in the cell dispersion. In vascular smooth muscle cells, Ca^{2+} currents have been characterized using single channel recordings in rabbit mesenteric artery (25), canine saphenous vein (26), and rabbit ear artery (27). Studies using whole-cell voltage clamp technique have been performed on rat mesenteric artery (28), rat portal vein (29), rabbit ear artery (27), and rat aortic smooth muscle cells (30).

Multiple types of calcium channels have been reported in a variety of excitable cells (31). At least three different calcium channels have been identified in dorsal root ganglion cells (32, 33), a large conductance channel that gives rise to a long-lasting current at positive membrane potentials (L type), a transient, smaller conductance channel that activates at more negative potentials (T type), and one with transient kinetics at positive potentials (N type). Single Ca^{2+} channel activities also tend to deteriorate and disappear in excised patches, prompting the suggestion that cytoplasmic factors may play an important role in maintaining the function and integrity of the channel.

Two types of calcium channels have been found in a variety of mammalian arteries (27, 28, 34, 35) and veins (29, 36, 37). The first type is a low threshold, rapidly inactivating T-type channel with a unitary conductance of approximately 8 pS with Ba^{2+} as the permeant ion. Because the conductance of Ca^{2+} channels is normally very small, isotonic Ba^{2+} is often used in the recording pipette to maximize the single-channel conductance. This T-type channel passes Ba^{2+} and Ca^{2+}

equally well and is relatively insensitive to dihydropyridines. A high threshold L-type channel with a unitary conductance of 20–25 pS is also present which inactivates more slowly. The latter channel is more permeant to Ba^{2+} than Ca^{2+}, and is highly sensitive to dihydropyridine blockers and agonists. Two components of inward Ca^{2+} current, a low threshold current, I_{low}, and a high threshold current, I_{high}, were observed from whole-cell recordings in rat mesenteric artery smooth muscle cells (28). From the inactivation properties of these macroscopic currents, the smaller conductance T-type channel is proposed to be responsible for I_{low} and the second larger conductance L-type channel is believed to give rise to I_{high} (26–28).

Multiple Ca^{2+} channels are also present in mammalian intestinal smooth muscle cells. Two types of voltage-dependent Ca^{2+} channels with different single-channel conductances and inactivation kinetics were reported from cell-attached patch-clamp recordings from guinea pig taenia coli (38). One channel has a conductance of 25 pS and a threshold of activation near −40 mV. When multiple current traces are summed, the mean current obtained inactivated slowly. The second Ca^{2+} channel, with a smaller conductance of 12 pS, displayed rapid inactivation. The large conductance channel alone was selectively blocked by the dihydropyridine derivative, nifedipine. The predominant Ca^{2+} current in visceral and myometrial smooth muscle cells is the larger conductance channel which is similar to the L-type current found in cardiac and vascular muscle, with a fairly high activation threshold, Ca-dependent inactivation and sensitivity to dihydropyridines (39, 40). The properties of the smaller conductance channel appear to be similar to those of the T-type Ca^{2+} channel in neuronal and cardiac cells. Thus, similar types of Ca^{2+} channels are present in gastrointestinal and vascular smooth muscle cells.

The general consensus is that each channel type contributes to different physiologic functions. Since the low voltage-activated T-type Ca^{2+} channel is activated near the resting potential level of vascular smooth muscle cells, it might play an important role in the control of intracellular Ca^{2+}-dependent processes such as regulation of vascular tone. The larger L-type channel is believed to be responsible for the depolarization phase of the action potential.

To study whether external Ba^{2+} has an effect on the gating properties of Ca^{2+} channels, Aaronson *et al.* (41) have used the whole-cell patch-clamp technique to characterize the inward Ca^{2+} current in freshly isolated cells from the rabbit ear artery in bathing solutions containing a physiologic concentration of Ca^{2+} or high Ba^{2+}. In comparing the results obtained in normal Ca^{2+} and high Ba^{2+} solutions, a shift of approximately 25 mV in the membrane potential at which the current was half-inactivated was found, indicating that Ba^{2+} does indeed alter the voltage dependence of the gating process.

Modulation of Ca^{2+} currents by agonists, presumably via channel protein phosphorylation, has been shown in a variety of cell types (42). In amphibian gastric smooth muscle, acetylcholine and substance P increase the depolarization-activated inward current through L-type Ca^{2+} channels (43). It has been suggested that this stimulatory effect may be mediated by the phosphoinositide metabolite diacylglycerol (43). These effects will be further discussed in later sections.

Sodium Channels. Although calcium channels are responsible for the depolarization phase of smooth muscle action potentials, fast inward sodium currents have been reported from cultured vascular smooth muscle cells dissociated from azygous veins of neonatal rats (37) and rabbit pulmonary artery (44). These two sodium channels, however, differ in their TTX sensitivity. The sodium current from rabbit artery was highly sensitive to nanomolar concentrations of TTX, while those from azygous veins were only blocked in the presence of micromolar concentrations of TTX. Thus far, there have been no reports of the presence of voltage-gated Na^{+} channels in dissociated gastrointestinal smooth muscle cells. The physiologic functions of these Na^{2+} channels remain to be determined.

Potassium Channels. In many smooth muscle cells, the major outward current in response to membrane depolarization is carried by Ca^{2+}-activated K^{+} channels (16, 18, 23, 45).

The Ca^{2+}-activated K^{+} channel has been reported from the following cultured smooth muscle cells: toad stomach (18, 46–49); three different species of frog stomach, *Rana esculenta*, *Rana temporaria* (48), and *Rana pipiens* (50); Xenopus stomach (48); rabbit jejunum (45, 51); rabbit longitudinal colonic muscle (23); rabbit mesenteric artery (45); rabbit portal vein (52, 53); guinea pig stomach (16); guinea pig taenia coli (54, 55); guinea pig mesenteric artery (45); rat pancreas vascular fragments (56); rat aorta (57); canine colonic myocytes (58) and canine coronary arteries (59).

The properties of Ca^{2+}-activated K^{+} channels in a variety of cell types have recently been reviewed (60). One of the best studied Ca^{2+}-activated K^{+} channels is that recorded from freshly dissociated vertebrate smooth muscle cells from the stomach of *B. marinus* (46, 47). It is highly selective for K^{+} and has a single-channel conductance in the order of 250 pS when bathed in symmetrical 130 m*M* KCl. With physiologic K^{+} gradient across the patch, the conductance is approximately 100 pS. The probability of the channel being in the open state (P_o) increases with increasing concentrations of intracellular Ca^{2+} as well as membrane depolarization. Both the mean closed time and the mean open time vary with membrane potential and can be fitted by single exponentials. The authors attrib-

uted all of the voltage sensitivity of P_o to reside in the mean closed time. This is in contrast to studies in other smooth muscle cells which have attributed the voltage dependence to the Ca^{2+} binding steps (61, 62). The rectification of the channel conductance observed in the presence of asymmetrical K^+ solutions has been attributed to internal Na^+ ions interfering with the movement of K^+ ions through the open channel (63). Second messengers may also play a role in modulating Ca^{2+}-activated K^+ channels. For example, GMP has been demonstrated to modulate Ca^{2+}-activated K^+ channels in excised inside-out patches of bovine aortic smooth muscle cells by increasing the probability of opening and the mean open time (64). The ubiquitous nature of this channel and its properties suggest that it is responsible for the large peak outward macroscopic current found in whole-cell voltage clamp studies.

Procaine, Ba^{2+}, tetraethylammonium (TEA), and quinidine are some of the chemical agents that have been found to inhibit current flow through the Ca^{2+}-activated K^+ channels. Benham *et al.* (51) proposed that the binding site for Ba^{2+} is approximately 80% of the way into the channel, whereas the binding sites for TEA and procaine are at or near the entrance of the channel. Applications of quinidine to dissociated gastric smooth muscle cells resulted in resolvable rapid flickerings between the open and blocked states of Ca^{2+}-activated K^+ channels with a corresponding reduction in open channel amplitude and an increase in open channel noise (65). Quinidine appears to have diverse mechanisms of action on potassium-selective channels in smooth muscle cells. It can act as a slow blocker on some channels and as an intermediate or fast blocker on others. Charybdotoxin, a protein purified from scorpion venom, has been found to be a potent specific blocker of the large conductance Ca^{2+}-activated K^+ channel in a variety of cell types (66, 67).

A smaller Ca^{2+}-activated K^+ channel with a slope conductance of 135 pS in symmetrical 142 m*M* KCl solutions was observed from cultured smooth muscle cells of the rat aorta (57). The channel opening probability increased when the intracellular calcium concentration was increased over 10^{-7} *M* or when the membrane was depolarized. The channel was blocked by either external TEA (10–30 m*M*) or by internal barium (1–5 m*M*).

In addition to the large conductance K^+ channel, another K^+-selective channel has been identified in longitudinal smooth muscle cells derived from rabbit jejunum (68, 69). These channels were also activated by membrane depolarization and have a smaller single-channel conductance of approximately 50 pS. However, in contrast to the large conductance channel, this channel appears not to be affected by changes in the concentration of intracellular calcium, $[Ca^{2+}]_i$. It has slower channel kinetics of the order of seconds and rectifies strongly in response to voltage. Since this K^+ channel is activated only during prolonged depolarization, it is unlikely that this channel plays a role in the repolarization of the membrane following an action potential. However, it may also be opened either during prolonged bursts of spike activity, during drug-induced depolarizations, or during slow depolarizations such as slow wave activity commonly observed in intact intestinal smooth muscle. A potassium-selective channel that is apparently unaffected by $[Ca^{2+}]_i$ and has a conductance around 70 pS has also been described in membranes of smooth muscle cells of frog and toad stomach (48).

Time-dependent whole-cell K^+ currents have been described in cells from rabbit pulmonary artery (70), toad stomach (3, 18), rabbit and guinea pig stomach (16), rabbit jejunum (71), and cat and rabbit colon (24). In some of these preparations, K^+ current appears to be partially activated by influx of Ca^{2+} through voltage-activated Ca^{2+} channels. Another type of K^+ channel that carries a spontaneous transient outward current has been observed in rabbit jejunum and ear artery cells (14) and in intestinal smooth muscle ball cells (72). These transient K^+ currents appear to be activated by quantal release of Ca^{2+} from internal stores.

Whole-cell voltage clamp studies on isolated smooth muscle cells of rabbit portal vein revealed the presence of four distinct K^+ currents: a time-independent background current, a slowly activating delayed rectifier, a smooth transient outward current, and an oscillatory spontaneous transient outward current (73). The time-independent background current is similar to that described by Inoue *et al.* (74) of a K_M channel in rabbit portal vein with a slope conductance of 180 pS in symmetrical 142 m*M* K^+ solutions in excised membrane patches of the same preparation. This channel is preferentially blocked by intracellular TEA and is exclusively sensitive to the external Ca^{2+} concentration. This channel may be responsible for the resting membrane potential in these cells. The delayed outward current has not been studied as extensively as Ca^{2+}-activated K^+ channels in isolated smooth muscle cells. A component of macroscopic current that appears to be insensitive to calcium has been reported but not characterized in rabbit ileal cells (72) and aortic smooth muscle cells in culture (75). The delayed rectifier described by Hume and Leblanc (73) shared similarities with a 50 pS K^+ channel identified by Benham and Bolton (68) in longitudinal smooth muscle cells of rabbit jejunum. Both are blocked by TEA, insensitive to Ca^{2+}, display voltage-dependent gating behavior, and show little or no inactivation. The delayed rectifier is probably the main current responsible for membrane repolarization when the intracellular Ca^{2+} concentration is low. Both transient outward currents are activated by an increase in $[Ca^{2+}]_i$. The oscillatory type of

transient outward current appears to be related to a sudden release of Ca^{2+} from internal stores. On the other hand, the smooth transient outward current seems to depend more on the normal resting level of intracellular free calcium. This distinction is based on the observation that caffeine abolished only spontaneous transient outward currents when activity was present under control conditions (73). Both of these currents would be important for membrane repolarization when the intracellular Ca^{2+} concentration rises.

By using similar rabbit portal vein cells, Beech and Bolton (53) found that the outward potassium current activated upon depolarization can be separated into two components: one carried by large conductance Ca^{2+}-activated K^+ channels (I_{cK}) and the other by channels whose characteristics resemble those of delayed rectifier channels (I_{dK}) seen in other excitable tissues. I_{cK} did not inactivate and was completely blocked by TEA. The inactivation of I_{dK} was slow and appeared to have two components. Slowly inactivating K^+ currents similar to I_{dK} have been reported for single smooth muscle cells from rabbit pulmonary artery (70). From single-channel recordings with a physiologic K^+ gradient, single-channel conductances of about 100 pS and 5 pS were reported for I_{cK} and I_{dK}, respectively. The results suggest that outward current evoked by depolarization from the resting potential is carried by both of these channels, thus contributing to the repolarization phase of the action potential in this smooth muscle.

Transient and oscillatory outward currents (I_{TO} and I_{OO}) can be recorded in membrane from the longitudinal smooth muscle cells of the rabbit ileum (72). The amplitude of I_{TO} and the activity of I_{OO} depend on the membrane potential and are closely related to $[Ca^{2+}]_i$, thereby suggesting that the ionic nature of I_{TO} and I_{OO} is through Ca^{2+}-dependent K^+ channels. Bath application of TEA completely blocked both currents.

Chloride Channels. Chloride channels have been identified in smooth muscle cells from a rat common carotid artery clonal cell line using the patch-clamp technique (76). Two different types of chloride channels were found: a large flickering channel with a single-channel conductance >150 pS which was activated by membrane depolarization and a small flickering channel with a single-channel conductance of 12 pS. Another large conductance voltage-activated Cl^- channel has also been reported from cultured rat aorta smooth muscle cells (77). This latter channel was found to be activated by protein kinase C inhibitor, suggesting that phosphokinase C may play a role in the regulation of this channel (78). Several chloride-selective channels with different single-channel conductances have also been observed in freshly dissociated frog stomach cells (79). The physiologic functions of these Cl^- channels remain to be studied.

Effects of Various Chemicals on Channels

Some of the channels described previously are also modulated by chemicals acting as transmitters or second messengers. The effects of some of these agents will be described in the following sections.

Adenosine Triphosphate. There is considerable evidence that ATP is an excitatory sympathetic cotransmitter in some arteries and other smooth muscles such as vas deferens (80). ATP-activated channels in vascular smooth muscle are cation selective (81) and the channels are directly gated by ATP without the involvement of any intermediate steps (82). These channels exhibit a selectivity for Ca^{2+} of three to one over Na^+. Working with single smooth muscle cells dissociated from rabbit ear artery, Benham (83) concluded that ATP-gated channels admit sufficient Ca^{2+} even in a physiologic Ca^{2+} gradient to significantly elevate intracellular Ca^{2+}. About 10% of the ATP-gated current may be attributed to Ca^{2+} ions.

ATP has also been proposed to play an important role in the regulation of the Ca^{2+} channel in smooth muscle cells (84, 85). In single smooth muscle cells dissociated from guinea pig portal vein, the presence of intracellular ATP was demonstrated to be required for the activation of single slow L-type Ca^{2+} channels (84). The major effect of ATP appeared to be either a change in the number of functional Ca^{2+} channels within the patch or an increase in the opening probability of the channel. The current amplitude or the mean open time of the channels was not affected. Thus, ATP-activated channels may have a dual excitatory function. The influx of Na^+ through cation-selective channels leads to membrane depolarization and action potential discharge. Increases in intracellular Ca^{2+} by direct entry of Ca^{2+} through the ATP-activated channels and voltage-gated Ca^{2+} channels may then activate contractile proteins as well as other Ca^{2+}-dependent enzymes modulating the contractile process.

Hyperpolarizing vasodilators such as cromakalim and pinacidil were found to activate ATP-sensitive K^+ channels in smooth muscle cells dissociated from rabbit or rat mesenteric arteries (86) with a single-channel conductance of 135 pS. Cromakalim and pinacidil have been shown to relax airway, intestinal and uterine smooth muscle, suggesting that the ATP-sensitive K^+ channel exists in many types of smooth muscle. The ATP-sensitive K^+ channel may provide a link between metabolism and the regulation of blood flow through its ATP-dependence.

Acetylcholine. Acetylcholine (ACh) causes contraction in a variety of gastrointestinal smooth muscle cells by binding to muscarinic receptors leading to action potential generation. It also prolongs the plateau potential in gastric muscle and induces spike potentials in small intestinal muscle (6). High doses of acetylcho-

line have been shown to suppress spontaneous currents through Ca^{2+}-activated K^+ channels in toad stomach (87).

Differing results have been reported in describing the mechanism underlying the action of acetylcholine for gastric and intestinal smooth muscle cells. In dissociated toad gastric smooth muscle cells, it appears that acetylcholine depolarizes the membrane by switching off a resting membrane conductance to K^+ (88, 89). This observation is strikingly similar to the potassium-selective M current that is suppressed by muscarinic agonists in sympathetic ganglia (90). This channel is inactive at the resting membrane potential but is activated within tens of milliseconds after depolarization. Acetylcholine, however, also has a second action on these cells. Clapp *et al.* (91) observed an increase in the amplitude and slowing of the decay of voltage-activated Ca^{2+} current. In these toad gastric smooth muscle cells, acetylcholine was found to increase the high threshold Ca^{2+} current but not the low threshold Ca^{2+} current (92). The latter effect can be mimicked by the addition of a diacylglycerol analog, suggesting that diacylglycerol may act as a second messenger in the acetylcholine response, presumably via activation of phosphokinase C.

In mammalian small intestine smooth muscle cells, the mechanism of action of muscarinic agonists appears to be different. Working with enzymatically dispersed smooth muscle cells from the longitudinal muscle of the rabbit jejunum in the whole-cell recording configuration, Benham *et al.* (93) found that acetylcholine activates a nonselective voltage-sensitive inward current. These nonselective cation channels are different from the Ca^{2+}-dependent ones reported in neuroblastoma (94) and are activated by muscarinic receptors. The sustained depolarization can induce Ca^{2+} influx via voltage-activated Ca^{2+} channels (93, 95). The channels select poorly between Na^+ and K^+, have a single-channel conductance of 20–30 pS, and are voltage dependent (95). Voltage-dependent gating of these channels has been suggested from the time-dependent decay of the ACh-induced inward current (93). Inoue and Isenberg (96) recently described the detailed feature of voltage-dependent gating of whole-cell currents through ACh-activated nonselective cation channels in guinea pig ileum smooth muscle cells. Any possible contribution of K^+ and Cl^- currents in these cells was greatly suppressed by loading the cells with cesium aspartate solution via the patch electrode. The channel shows a strong voltage dependence with the opening probability increasing with membrane depolarization. This voltage dependence can act as a positive feedback mechanism to prolong the depolarizing effects of ACh, ultimately leading to the initiation of spikes and hence contraction.

Norepinephrine. Norepinephrine has powerful effects on the electrical and mechanical activity of vascular smooth muscle cells. Experiments on a variety of vascular smooth muscle preparations suggest that norepinephrine acts through multiple mechanisms (97). Norepinephrine can promote Ca^{2+} delivery via mechanisms that do not require membrane depolarization. Another possibility is that norepinephrine can enhance Ca^{2+} current through voltage-gated Ca^{2+} channels. In smooth muscle cells dissociated from rabbit mesenteric arteries, norepinephrine was found to increase the probability of opening of voltage-gated Ca^{2+} channels (98). In single rabbit ear artery smooth muscle cells (99), micromolar concentrations of norepinephrine were also found to produce an increase in the dihydropyridine-sensitive L-type Ca^{2+} currents, without significantly affecting the time and voltage dependence of channel activation and inactivation. The dihydropyridine-insensitive T-type Ca^{2+} current in these cells was not affected. However, the T-type Ca^{2+} currents in portal vein cells have been reported to be enhanced by norepinephrine (100). Because norepinephrine's effects on ear artery cells were not inhibited nor mimicked by traditional α- and β-antagonists and agonists, the action of norepinephrine on these smooth muscle cells was hypothesized to occur through interaction with an adrenergic receptor different from the conventional α- and β-adrenergic receptors. Preliminary results suggest the involvement of GTP-binding proteins as a coupling mechanism between agonist binding and channel modulation. The modulation of L-type Ca^{2+} channels by norepinephrine may play an especially important role in promoting sympathetic vasoconstriction in resistance blood vessels where Ca^{2+} stores are relatively poorly developed. In rabbit portal vein smooth muscle cells (101), norepinephrine was found to open a Cl^--selective channel and a cation-selective channel. Recently, another report using isolated rat portal vein cells supported the observation that the norepinephrine-induced depolarization is mediated by an increase in chloride conductance. The increase, however, is activated by an increase in intracellular Ca^{2+} either through the release from internal stores or via voltage-gated Ca^{2+} channels (102).

Arachidonic Acid. In freshly dissociated toad stomach smooth muscle cells, arachidonic acid, as well as fatty acids that are not substrates for cyclooxygenase and lipoxygenase enzymes, activated a specific type of potassium channel (103). In the whole-cell configuration, micromolar concentrations of arachidonic acid rapidly and reversibly activated an outwardly rectifying K^+ current. Fatty acids may, therefore, have an important regulatory role in the function of ion channels in smooth muscle. The activation of K^+ channels by fatty acids in excised patches occurred in the absence of Ca^{2+} and nucleotides, suggesting that fatty acids themselves may act directly at a protein or lipid site in the mem-

brane. In smooth muscle cells, an increase in fatty acids would, therefore, lead to membrane hyperpolarization and a subsequent reduction in contractile activity. In human aorta smooth muscle cells, the fatty acid, 2-decanoic acid, was found to increase dramatically the probability of opening of Ca^{2+}-activated K^+ channels (104). In addition to being activated by Ca^{2+}, the channel was also found to be activated by normally ineffective Mg^{2+} ions, suggesting that the fatty acid can alter the Ca^{2+}-binding specificity of the channel.

Substance P. Substance P is released by motoneurons innervating gastrointestinal muscle and has been hypothesized to act as an excitatory transmitter (105). Working with rabbit longitudinal colonic muscle, Mayer *et al.* (23) observed that subnanomolar concentrations of substance P increased the opening probability of Ca^{2+}-activated K^+ channels. Their data demonstrated, however, that this increase is an indirect effect, with substance P acting primarily through an increase in Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels by binding to neurokinin NK-1 receptors. This hypothesis is supported by the observation in toad gastric muscle (43, 106) of a stimulatory effect of substance P on dihydropyridine-sensitive Ca^{2+} currents.

In toad gastric smooth muscle cells, substance P was also found to inhibit an acetylcholine-sensitive K^+ current (87). However, the effects of substance P appear to act through a receptor that is distinct from the muscarinic receptor.

Neurokinin A (NKA), a neuropeptide that has similar characteristics and pharmacologic properties as substance P, is known to produce contraction in smooth muscles. In rat vas deferens, NKA, but not substance P, was found to suppress a voltage-gated outward K^+ current (107). The inhibition of the K^+ current in toad stomach resulted in depolarization and generation of action potentials, while the suppression of the K^+ current in this study resulted in prolongation of action potentials. Theoretically, this can increase Ca^{2+} influx through voltage-gated Ca^{2+} channels and contribute, at least partially, to the NKA-induced contractions. Further studies are necessary to determine the relationship between the NKA-sensitive K^+ current and the substance P-sensitive current in toad stomach.

Inositol Triphosphate. In planar lipid bilayers into which vesicles made from aortic muscle sarcoplasmic reticulum were incorporated (108), inositol 1,4,5-triphosphate (IP_3) was found to induce openings of Ca^{2+} channels with a single-channel conductance of approximately 10 pS.

The effects of IP_3 have been observed on the inward and outward currents of dispersed smooth muscle cell of the guinea pig portal vein (15). The Ca^{2+} inward current was slightly reduced but the frequency of the oscillatory outward current was enhanced, with no change in the sustained outward current. The main action of IP_3 is to increase the frequency of release of Ca^{2+} from the sarcoplasmic reticulum rather than the amount of Ca^{2+} released during each event. The released Ca^{2+} also activates the Ca^{2+}-dependent K^+ channel and forms transient and oscillatory outward currents.

Agonist-stimulated release of calcium from internal stores is believed to be brought about by increased production of IP_3 due to the accelerated activity of a triphosphoinositide phosphodiesterase. Control of this enzyme may involve a guanine nucleotide-binding protein (G protein). G proteins have been implicated in the transduction of signals from several types of receptor including muscarinic receptors. With the use of single dispersed cells from longitudinal muscle of rabbit small intestine, Bolton and Lim (109) monitored the discharge of spontaneous transient outward currents which they believed to correlate with the sporadic release of calcium from internal stores. These spontaneous transient outward currents were quickly abolished in cells with patch electrodes filled with nonhydrolyzable analogs of GTP such as GTPγS or Gpp(NH)p but were large or normal in size in cells where GDPβS, a GTP analog, was used in the pipette. The introduction of GTPγS or Gpp(NH)p into the cell also inhibited outward current in response to caffeine or carbachol, but had no effect on calcium-activated potassium channel activity. These results suggest that guanine nucleotide analogs are acting on the internal calcium store rather than on calcium-activated potassium channels.

Angiotensin II. One of the most potent vasoconstrictors of vascular vessels is the peptide angiotensin II. Binding of angiotensin II to its receptor activates the formation of IP_3 and 1,2-diacylglycerol through a G protein-mediated activation of phospholipase C. The observed rise in intracellular Ca^{2+} is believed to be caused by an IP_3-induced Ca^{2+} release from the endoplasmic reticulum. The increase in internal Ca^{2+} in turn triggers contraction. In a study with coronary smooth muscle membrane vesicles incorporated into lipid bilayers, angiotensin II was found to inhibit Ca^{2+}-activated K^+ channels by changing the gating process and producing a long blocked state (110). A direct inhibition of Ca^{2+}-activated K^+ channels would then contribute to membrane depolarization and subsequent contraction.

Conclusion

Gastrointestinal and vascular smooth muscle cells appear to possess similar ion channels in their membranes but differ in how some of these channels are modulated. This, however, is not surprising since they react in a different manner physiologically to autonomic neurotransmitters such as acetylcholine and norepinephrine. Smooth muscle constrictors, norepineph-

rine in the case of vascular muscle, and acetylcholine in the case of gastrointestinal muscle, appear to share the common property of activating Ca^{2+} channels. However, whether these agents affect the Ca^{2+} channels directly or through second messenger systems remain to be determined. Vasodilators, on the other hand, appear to work via activation of K^+ channels, specifically, the ATP-sensitive K^+ channel. The ionic mechanism responsible for norepinephrine's decrease in the motility of gastrointestinal smooth muscle cells remains unknown at the present time.

Thus, a wealth of information has been gathered within the past several years concerning the ionic channels that are present in various types of dissociated smooth muscle cells utilizing the patch-clamp technique. However, a lot of important questions still remain unanswered and will no doubt be an area of fruitful research for the foreseeable future. Another challenge that needs to be addressed in the future would be to apply the knowledge learned from these isolated cells to account for the observations seen in intact tissue.

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