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Critical Review

Role of Interstitial Cells of Cajal in the Control of Gastric Motility

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Abstract. Most regions of the gastrointestinal tract generate spontaneous electrical and mechanical activity in the absence of stimulation. When electrical recordings are made from slow muscle cells lying in the gastrointestinal tract, a regular discharge of long lasting waves of depolarization, slow waves, is detected. It has recently become apparent that slow waves are generated by a specialized population of smooth muscle cells, known as interstitial cells of Cajal (ICC). ICC can be subdivided into at least two separate groups. In most regions of the gastrointestinal tract, one group of ICC form a network that generates pacemaker potentials, so producing rhythmical membrane potential changes in the adjacent muscle layers. The second group of ICC are distributed amongst the smooth muscle cells and are tightly electrically coupled to them. In some regions of the gut, the second group of ICC augment the waves of pacemaker depolarization, so ensuring that voltage-dependent calcium channels in the smooth muscles are activated during each slow wave cycle. In addition, the second group of ICC are densely innervated by inhibitory and excitatory nerve terminals. Thus intrinsic nerve terminals, rather than communicating directly with smooth muscle cells, selectively innervate ICC and release transmitters directly onto them. The signals that are generated in the ICC, by the neurally released transmitters, then alter the activity of surrounding smooth muscle cells.

Keywords: smooth muscle, slow wave, interstitial cell

Interstitial cells of Cajal and the control of gastric motility

The control of gut motility resides in a balance between myogenic, neuronal, and hormonal factors. When isolated most, but not all, regions of the gastrointestinal tract generate rhythmical mechanical activity in the absence of neuronal or hormonal stimulation. When electrical recordings are made from the muscle layers of myogenically active regions of the gut, rhythmical waves of depolarization, termed slow waves, are recorded from the smooth muscle cells. Each slow wave triggers a contraction, but although the contractions are blocked by agents that block smooth muscle voltage-dependent calcium channels, the amplitudes and time courses of slow waves are little changed. Since slow waves were readily recorded from smooth muscle cells, it was thought that slow waves were generated by them (1). However when single smooth muscle cells were isolated from many different regions of the gut, it became apparent that they were unable to generate slow waves nor did they possess the ion channels necessary to generate such activity (2). Subsequently it was recognized that the muscular wall of the gastrointestinal tract, as well as containing smooth muscle cells, contained a set of specialized cells that lacked contractile elements. These cells are termed Interstitial Cells of Cajal (ICC) and it was suggested, largely from structural studies, that these cells might be pacemaker cells (3). In general ICC can be divided into two groups. In most regions of the gastrointestinal tract, a thin layer of ICC forms a network of cells lying between the longitudinal and circular layers in the myenteric region, ICC_{MY}. The second group of ICC has an intramuscular location (ICC_{IM}), with individual ICC being distributed amongst the smooth muscle cells making up the muscle layers. The distribution of ICC_{IM} shows regional variation. In the small intestine, ICC_{IM} are concentrated in the deep circular muscle layer (ICC_{DMP}, ref. 4). In the gastric

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antrum, ICC_{IM} are widely distributed throughout the circular layer (4, 5) and only very few are found in the longitudinal layer (6). In the fundus, a myenteric network of ICC is absent, but ICC_{IM} are widely distributed through both the circular and longitudinal muscle layers (4).

Direct evidence for the importance of ICC in the generation of slow waves came from experiments examining the small intestines of white-spotting (*W*) mutant mice, in which the development of ICC is impaired. In the heterozygote W/W^V strain, ICC_{IM} are present in the small intestine, ICC_{MY} are absent: in the homozygote, where all ICC are absent, the mutation is lethal. The small intestines of wild type mice generate slow waves whereas those of W/W^V mice fail to do so (7, 8). The essential role of ICC_{MY} was further supported by experiments where the development of ICC was blocked. ICC express a tyrosine kinase receptor, Kit. When this receptor was inactivated by treating with an antibody to Kit, ICC failed to develop and the preparations failed to generate slow waves (9). In addition, isolated ICC_{MY}, unlike smooth muscle cells, were found to spontaneously generate electrical activity (10, 11). The role of ICC_{MY} in the generation of slow waves was further clarified by making recordings from them *in situ*. In preparations of guinea pig antrum, ICC_{MY}, identified by dye injection, were found to generate large amplitude, long-lasting pacemaker potentials (Fig. 1); these occurred at the same frequency as did slow waves recorded simultaneously from the nearby circular layer (12). Similarly when simultaneous recordings were made from ICC_{MY} and the longitudinal layer, both waves of depolarization again occurred synchronously (13). Critically in both sets of experiments, the onset of pacemaker depolarization generated by ICC_{MY} preceded the onset of depolarization in either muscle layer. Furthermore, ICC_{MY} are electrically coupled to the adjacent muscle layers, so allowing the passive flow of pacemaker current into both muscle layers (6). Together the observations indicate that electrical activity is initiated by ICC_{MY}, with each pacemaker potential passively depolarizing the adjacent muscle layers (14).

In the circular muscle layer, each passive wave of depolarization, originating in ICC_{MY}, is augmented by a secondary regenerative component (Fig. 1, ref. 15). The secondary component of the slow waves is also initiated by ICC rather than smooth muscle cells. As has been pointed out, the small intestines of W/W^V mice lack ICC_{MY} but contain ICC_{IM}. In the antrum, the situation is reversed, ICC_{MY} are present whereas ICC_{IM} are absent (16). In the antrum of W/W^V mice rhythmical electrical activity was detected, but unlike the slow waves recorded from wild type mice, the waves of depolarization lack secondary regenerative components (17, 18). Thus, in the antrum, the primary component of each slow wave is initiated by ICC_{MY}. The passive wave of pacemaker depolarization is then augmented by the secondary component, initiated by ICC_{IM}. Each antral slow wave therefore reflects the sum of contributions made by ICC_{MY} and ICC_{IM}. The contributions made by ICC_{MY} and ICC_{IM} vary with location (Fig. 2). ICC_{MY} are abundant near the greater curvature but decrease in density to be almost completely absent at the lesser curvature (18). In the W/W^V mutant, where ICC_{IM} are absent, large primary components are detected at the greater curvature but these decrease in amplitude as the density of ICC_{MY} falls towards the lesser curvature (18). In the wild type mouse, where ICC_{IM} are present, slow

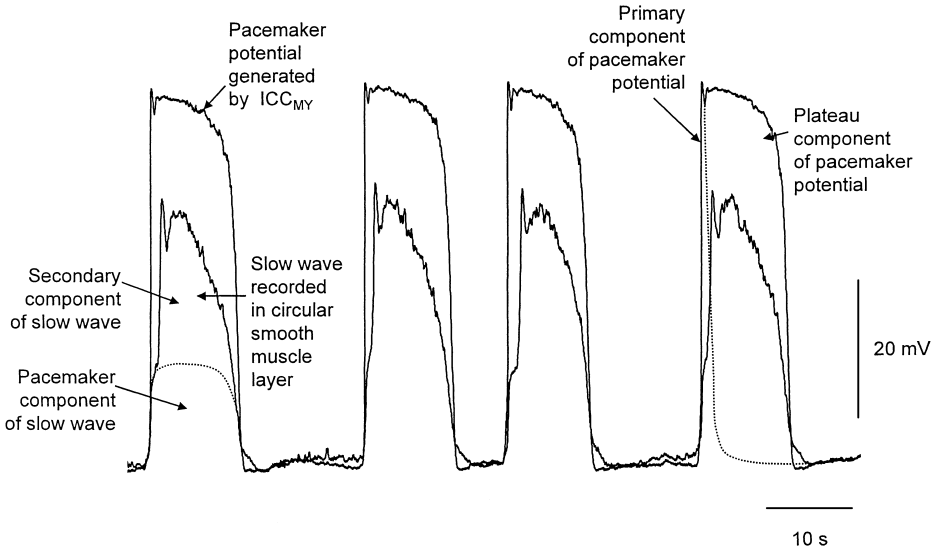

Fig. 1. Properties of pacemaker potentials and slow waves, recorded simultaneously from the gastric antrum of the guinea pig. It can be seen that each pacemaker potential, generated by ICC_{MY}, triggers a two component slow wave in the circular muscle layer. Each pacemaker potential is made up of two components, a brief primary component and a prolonged plateau component. Each slow wave starts with the passive pacemaker component which triggers the secondary component of the slow wave.

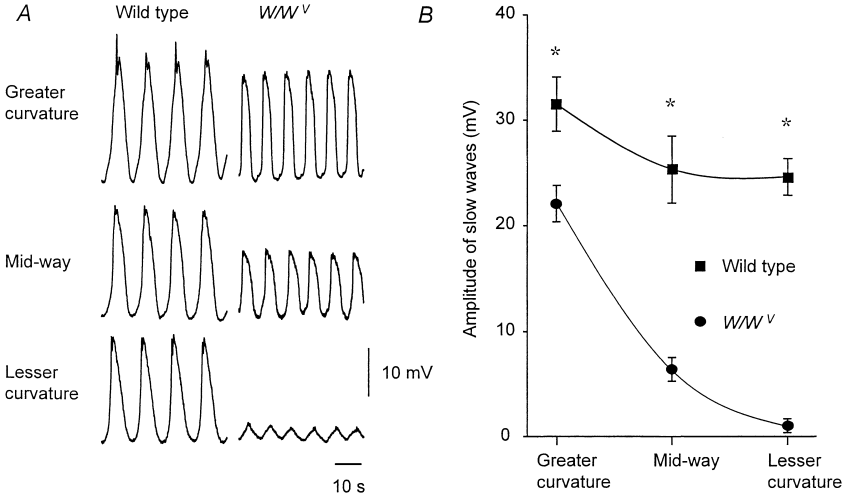

Fig. 2. Properties of slow waves, recorded from different regions of the gastric antrum of wild type and W/W^V mice. Slow waves were recorded from the greater curvature, midway between the greater and lesser curvature and from the lesser curvature. It can be seen that slow waves had similar amplitudes when recorded from each location in the wild type mouse (*A*). In contrast when recorded from W/W^V mice, which lack ICC_{IM}, the waves of depolarization had the largest amplitudes at the greater curvature and decreased in amplitude towards the lesser curvature (*A*). The results are plotted graphically in Fig. *B*; the stars indicated that the mean peak amplitudes of the slow waves detected in the wild type mice and W/W^V mice were significantly different. (Reproduced from Ref. 18, with permission by Blackwell Publishing Ltd.).

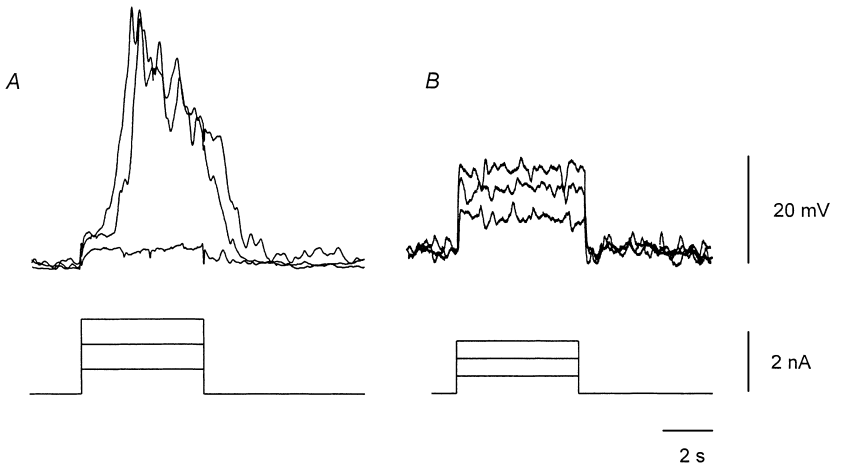

Fig. 3. Comparison between properties of bundles of antral and fundal circular muscle layer of the guinea pig. In each set of traces a single bundle of circular layer from either the antrum (*A*) or the fundus (*B*) was impaled with two independent sharp electrodes: one was used to pass current and the other to record membrane potential. It can be seen that when the antral bundle was depolarized above threshold it initiated regenerative responses (*A*) whereas although the fundal bundle displayed a characteristic discharge of membrane noise, depolarization failed to initiate regenerative responses (*B*).

waves have similar amplitudes in all regions, indicating that once a slow wave is initiated by ICC_{MY} at the greater curvature, ICC_{IM} support the generation of complete slow waves even in regions where ICC_{MY} are absent.

To directly test how the circular layer augments the waves of pacemaker depolarization spreading from ICC_{MY}, single bundles of antral circular muscle containing ICC_{IM} were isolated. Preparations were impaled with two independent electrodes. One electrode was used to pass depolarizing currents, so mimicking the waves of pacemaker depolarization normally produced by ICC_{MY}; the second electrode was used to record membrane potential changes. Depolarization triggered a response identical to the secondary component of the slow wave (5, 19). These responses were termed regenerative potentials (Fig. 3A). Regenerative potentials could not be initiated in bundles of circular muscle that lacked ICC_{IM}, confirming that they were initiated by ICC_{IM} (20). Although under normal circumstances pacemaker activity is initiated by ICC_{MY} in the antrum, in preparations where ICC_{MY} are dissected away, bundles of circular muscle containing ICC_{IM} continue to generate spontaneous myogenic activity, albeit at a frequency lower than when ICC_{MY} are attached (5, 21, 22). This indicates that ICC_{IM} themselves are capable of generating pacemaker activity but that they normally do so at a lower frequency than do ICC_{MY}. Hence in the intact antrum ICC_{MY} are the dominant pacemaker cells.

The fundus is not myogenically active (23) and although this might seem to simply result from the absence of ICC_{MY} in this tissue, both the longitudinal and circular muscle layers of the fundus contain ICC_{IM} (4). As pointed out, antral ICC_{IM} readily initiate spontaneous electrical activity (5, 21, 22) but clearly those of the fundus do not (23, 24).

Properties of myenteric interstitial cells of Cajal

How ICC_{MY} generate pacemaker potentials is not fully understood and is an area of controversy. It is generally agreed that the mechanisms by which ICC_{MY} generate pacemaker potentials differ from those used by cardiac pacemaker cells to generate cardiac pacemaker potentials. Cardiac pacemaker cells generate spontaneous activity through the sequential activation of several voltage-dependent ion channels along with a contribution from voltage-independent resting, or background, channels (25). In the antrum, pacemaker activity depends critically on the release of Ca^{2+} from IP_3-dependent internal stores. Thus preparations of gastric antrum from mutant mice that lack IP_3 type-1 receptors fail to generate slow waves (26).

When recordings have been made from ICC_{MY}, *in situ*, which lie either in the gastric antrum or in the small intestine, a common picture emerges. In both tissues, pacemaker potentials are made up of two pharmacologically distinct components (Fig. 1). Each pacemaker potential has a primary component, with rapid rise time and duration of about 1 s. The primary component is abolished when the external concentration of calcium ions ($[Ca^{2+}]_o$) is lowered or by nickel ions (13, 27, 28). Each primary component is followed by a plateau component that in the antrum lasts for some 5 to 10 s (13, 27) but in the small intestine only lasts for about 1 s (28). The plateau component is selectively abolished by caffeine and some chloride channel antagonists, but is little affected by reducing $[Ca^{2+}]_o$ (12, 13, 27, 28). To abolish rhythmical activity completely, both components must be abolished (27, 28). This suggests that there are two independent mechanisms that generate pacemaker activity, but that under physiological conditions, both occur in a coordinated manner. All ICC_{MY} examined to date generate an ongoing discharge of spontaneous small amplitude depolarizing potentials, termed unitary potentials (13, 28). Observations made on ICC_{MY} *in situ* suggest that each unitary potential results from the packeted release of Ca^{2+} from internal stores and the direct activation of sets of ion-selective channels in the membranes of ICC_{MY} (27). In gastric ICC_{MY}, the discharge of unitary potentials gradually increases in frequency in the interval between pacemaker potentials until the threshold for the primary component of the next pacemaker potential is reached. The primary component depolarizes the ICC_{MY} network and this dramatically accelerates the rate of discharge of unitary potentials, with the high frequency discharge of unitary potentials giving rise to the plateau component of the pacemaker potential. As the discharge of unitary potentials falls to a low level, the plateau component of the pacemaker potential terminates. The cycle is then repeated (13). Thus, the pacemaker depolarization is generated by low frequency discharge of unitary potentials and the plateau component is generated by a high frequency discharge of unitary potentials. The generation of pacemaker potentials occurs in a stochastic manner with the interval between each pacemaker potential varying from beat to beat (13). When two pacemaker potentials occur at a short interval, the second has a briefer plateau component; when two pacemaker potentials occur with a greater separation, the plateau component of the second pacemaker potential has a longer duration (13). As each pacemaker potential terminates, whatever its duration, the time course of repolarization is identical: why this phenomenon occurs is not understood. Whatever the case, the analysis implies that, as well as involving the release of calcium ions from internal stores, each pacemaker potential involves two separate voltage-dependent gated steps, namely, the initiation of the primary component of the pacemaker potential and the voltage-dependent acceleration of unitary potential frequency during the plateau component.

This analysis of pacemaker activity does not provide an explanation for the nature of the channels activated during pacemaker activity nor does it explain what causes the channels to be activated. The simplest explanation is that unitary potentials are triggered by the packeted release of Ca^{2+} from internal stores. Because pacemaker activity is absent in mutant mice lacking IP_3 type-1 receptors (26) and because slow waves are abolished by 2-APB, an inhibitor of Ca^{2+} release from IP_3-dependent stores (13), IP_3-dependent calcium stores must generate the packets of Ca^{2+}. Furthermore although it is clear that ryanodine-receptor-coupled stores are present in smooth muscle cells (29), it is not clear that such stores are present in ICC. Certainly ryanodine-dependent internal calcium stores do not appear to contribute to slow wave activity, with low or high concentrations of ryanodine having no effect on the generation of gastric slow waves (30). After Ca^{2+} is released from IP_3-dependent stores, it leads to the opening of sets of Ca^{2+}-sensitive, ion-selective channels. Pharmacological analyses of the properties of pacemaker potentials recorded *in situ* suggest that calcium-activated chloride-selective channels are involved. Thus the plateau component is transiently increased in amplitude when $[Cl^-]_o$ is reduced (13), whilst longer exposure to low $[Cl^-]_o$ solutions, which depletes the internal concentration of chloride ions ($[Cl^-]_i$) (31), abolishes the plateau component as do several blockers of Cl^- channels (27). Furthermore, Cl^--selective channels have been demonstrated in ICC freshly isolated

from the mouse small intestine (10). Thus the release of each packet of Ca^{2+} from an individual IP_3-dependent store may cause the generation of a unitary potential by allowing an efflux of Cl^-. During the interval between pacemaker potentials, the frequency of generation of unitary potentials increases until the threshold for the initiation of the next primary component is reached. Again the nature of the channels underlying the primary component of the pacemaker potential is not understood. Observations on the properties of ICC_{MY} *in situ* indicate that the channels are readily excited by field stimulation (32), have low thresholds and are readily inactivated by moderate membrane depolarization (27). As noted previously, the primary component is abolished by reducing $[Ca^{2+}]_o$ and by nickel ions (13, 27). All of these observations suggest that the channels share many properties with T-type Ca^{2+} channels demonstrated in other excitable cells: indeed such channels have been demonstrated in freshly isolated canine ICC (33). After each primary component, the frequency of discharge of unitary potentials increases dramatically to generate the plateau component. This increase in frequency, like that demonstrated by ICC_{IM} (19), occurs after a latency of 1 s (13). We suggest that depolarization, associated with the primary component, either increases the production of IP_3 inside ICC presumably using a pathway involving PLC (34) or increases the release of Ca^{2+} from IP_3-dependent stores. After this dramatic increase in frequency of unitary potentials, the pathway then becomes refractory and the discharge of unitary potentials ceases. As the refractory period wanes, the spontaneous generation of unitary potentials restarts and gradually increases in frequency until the next pacemaker potential is triggered. The handling of Ca^{2+} by mitochondria has been shown to play a central part in the generation of pacemaker potentials by ICC, slow wave activity being abolished by agents that interfere with Ca^{2+} accumulation by mitochondria (35). In the above scheme, a role for mitochondria is not prescribed; presumably mitochondria could either supply Ca^{2+} to refill IP_3-dependent stores or be involved in the reuptake of Ca^{2+} after its release from IP_3-dependent stores.

Experiments involving cultured ICC do not provide support for all aspects of the preceding hypothesis. The pacemaker signals generated by tissue cultured ICC (36) differ somewhat in time course from those recorded from intact tissues and why this is so is not clear. Patch recordings from cultured ICC suggest that they lack calcium-activated channels of any description; rather they indicate that ICC possess cation-selective channels that are activated when internal concentration of calcium ion ($[Ca^{2+}]_i$) is reduced to low levels (37). Secondly, although high threshold voltage-dependent Ca^{2+} channels were identified, low threshold voltage-dependent Ca^{2+} channels were not detected (38). However, studies on cultured ICC also suggest that pacemaker activity arises from the packeted release of Ca^{2+} from IP_3-dependent stores. In cultured ICC, changes in $[Ca^{2+}]_{i\text{-Mitochondria}}$ have been detected during pacemaker activity, and it has been suggested that the initial increase in $[Ca^{2+}]_i$ stimulates uptake of Ca^{2+} on all surfaces of the mitochondrion (35). Thus $[Ca^{2+}]_i$ in a restricted space close to the ICC membrane will be lowered and sets of cation-selective channels, which are closed at normal levels of $[Ca^{2+}]_i$, will open: the channels are auto-inhibited as Ca^{2+} flows through them. The depolarization produced by the open cation-selective channels depolarizes the ICC and then activates high threshold Ca^{2+} channels. The ensuing influx of Ca^{2+} is suggested to trigger the subsequent release of Ca^{2+} from IP_3-dependent stores, so restarting the cycle. The explanation for the difference between cultured and freshly isolated ICC is not known. Whatever the case, either hypothesis to explain pacemaker activity in ICC indicates the importance of release of Ca^{2+} from IP_3-dependent stores and the subsequent activation of ion-selective membrane channels, the key roles played by mitochondria and a step involving inward current flow via voltage-dependent channels.

Interstitial cells of Cajal and the neural control of gastric motility

ICC_{IM} play a key role in neural control of the gastrointestinal tract (39). It has long been recognized from structural studies that ICC_{IM} are densely innervated (40, 41). The importance of these observations became apparent when the responses to nerve stimulation were examined in the fundus of W/W^V mutant mice. In control animals, which contain ICC_{IM}, nerve stimulation evoked excitatory and inhibitory junction potentials (EJPs and IJPs) in the circular muscle layer. In the fundus of W/W^V mice, which lacks ICC_{IM}, nerve stimulation failed to evoke EJPs or IJPs (16). However in the W/W^V mouse fundus, both excitatory cholinergic and inhibitory nitrergic nerve terminals were present in normal numbers and cholinergic nerve terminals continued to release ACh (42). These observations were not limited to the fundus or the W/W^V mutant. Essentially the same observations were made in the antrum: nitrergic IJPs and cholinergic EJPs were readily detected in tissues taken from control animals but not in tissues taken from W/W^V animals (20). When neuroeffector transmission was examined in fundal tissues taken from a mouse with a different mutation that also led to the loss of ICC_{IM}, namely, Steel mutant mice

(*Sl/Sl*^{d} mice), similar observations were made: EJPs and IJPs were detected in tissues that contained ICC_{IM} but not in those which lacked ICC_{IM} (43).

Analyses of cholinergic-EJPs and nitrergic-IJPs in intact antral tissues also support the concept that excitatory and inhibitory nerve terminals functionally innervate ICC_{IM} rather than smooth muscle cells. In the circular layer of the antrum, cholinergic nerve stimulation evokes a response that is identical to that evoked by depolarizing ICC_{IM} (32). Furthermore, when ICC_{IM} are depolarized, they give rise to a regenerative response that is followed by a long lasting refractory period (5). When cholinergic nerves are stimulated during this refractory period, they fail to initiate a response; conversely, when ICC_{IM} are depolarized immediately after cholinergic nerve stimulation, depolarization fails to initiate a regenerative response (Fig. 4, ref. 32). The responses initiated by either direct depolarization or cholinergic nerve stimulation are abolished by low concentrations of caffeine, some Cl^- channel antagonists, and 2-APB (32). In the intact antrum, cholinergic nerve stimulation selectively excites ICC_{IM}, so initiating premature regenerative responses in the circular layer. These large depolarizations 'drive' ICC_{MY}, so increasing the frequency of slow waves (32) and the ability of cholinergic nerve stimulation to increase the frequency of slow waves is lost in tissues devoid of ICC_{IM} (44). Finally, when ACh is applied to gastrointestinal smooth muscle cells, it activates cation-selective channels (2); in the gastric antrum, this conductance is not activated by neurally released ACh, but rather the conductance activated involves anion-selective channels located on ICC_{IM} (32, 45). When NO or NO donors are applied to gastrointestinal smooth muscle cells, potassium-selective channels are activated (46). However neurally released NO suppresses the discharge of unitary potentials by ICC_{IM} (20, 47). Thus the neural release of NO triggers a small hyperpolarization or prevents the voltage-activated increase in unitary potentials that occurs during a regenerative potential via a pathway involving the formation of cyclic-GMP (Fig. 5).

However, a major paradox remains. In most gastrointestinal muscles, inhibitory nerves appear to release two transmitters, ATP, which gives rise to a rapid-IJP, and NO, which gives rise to a slow-IJP. In mutant animals that lack ICC_{IM}, although the nitrergic responses are absent, the purinergic responses persist (20). This observation could be explained if there were two different sets of intrinsic inhibitory nerves, one set that innervated smooth muscle and released ATP, with the second set innervating ICC_{IM} and releasing NO. However there is no evidence to support this view; rather, the contrary is thought to be the case (48). Even more puzzling, in relation to this paradox is the observation that purinergic IJPs are only detected in gastrointestinal muscles where ICC_{IM} are present, being absent in the longitudinal layers of gastric antrum and ileum where ICC_{IM} are either absent or present in very small numbers (49, 50).

Properties of intramuscular interstitial cells of Cajal

At the outset it should be pointed out there have been no studies on the biophysical properties of isolated gastrointestinal ICC_{IM}. Studies have been carried out on single isolated ICC-like cells obtained from the urethra (51) and several observations made on urethral ICC may also apply to gastrointestinal ICC_{IM}. What is known

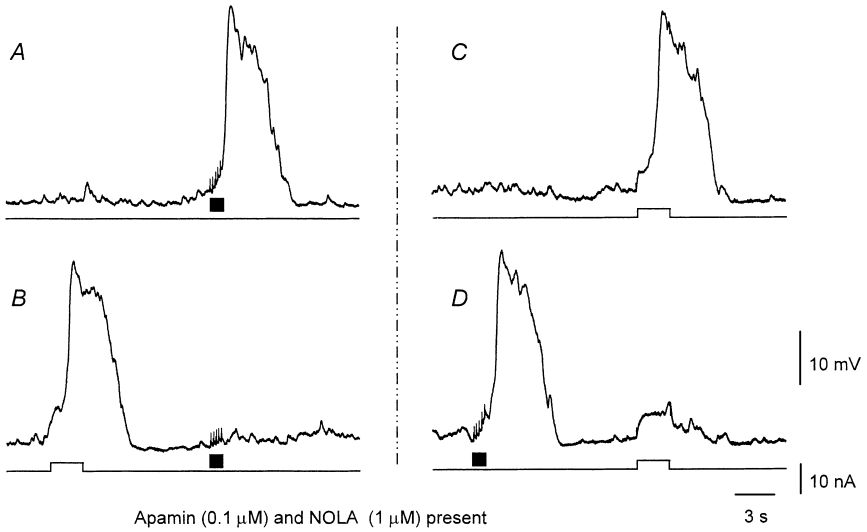

Fig. 4. Interaction between regenerative responses initiated by cholinergic nerve stimulation and membrane depolarization in a bundle of antral circular of the guinea pig. Cholinergic nerve stimulation and periods of membrane depolarization initiated very similar regenerative responses (*A* & *C*). However when excitatory nerves were stimulated in the refractory period following a regenerative potential triggered by depolarization, nerve stimulation was ineffective (*B*). Similarly membrane depolarization failed to initiate a regenerative response in the refractory period following a regenerative response triggered by excitatory nerve stimulation (*D*). Apamin and NOLA (L-nitroarginine) were present throughout to abolish the effects of concurrent inhibitory nerve stimulation. (Reproduced from Ref. 32, with permission by Blackwell Publishing Ltd.).

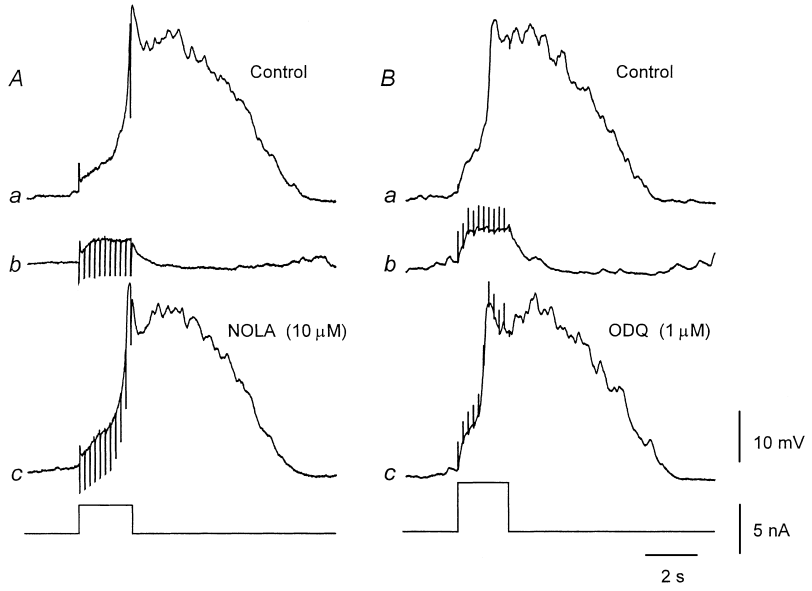

Fig. 5. Inhibition of regenerative responses by nitrergic nerve stimulation in a bundle of antral circular muscle of the guinea pig. A period of membrane depolarization initiated a regenerative response (*Aa*), which was inhibited by nitrergic nerve stimulation (*Ab*). After inhibiting the synthesis of NO, by treating the preparation with NOLA (L-nitroarginine), inhibitory nerve stimulation failed to inhibit the generation of regenerative responses (*Ac*). Nitrergic nerve stimulation inhibited regenerative responses by activating a second messenger pathway involving cyclic-GMP since the inhibition was blocked by ODQ (oxadiazolo quinoxalin-1-one), an agent which blocks cyclic-GMP formation (*Ba*, *b* & *c*). Atropine and apamin were present throughout to abolish the effects of concurrent excitatory nerve stimulation and the purinergic component of the inhibitory response. (Reproduced from Ref. 47, with permission by Blackwell Publishing Ltd.).

about the properties of gastrointestinal ICC_{IM} has been obtained from experiments carried out on intact preparations that contain sets of smooth muscle cells and ICC_{IM} electrically coupled together to form an electrical syncytium (14). In each of these studies, the contribution of smooth muscle cells to electrical signals generated by the syncytium has been minimized by blocking the voltage-dependent calcium channels known to be present in the smooth muscle cells (5, 19).

A characteristic property of the circular layer of the gastric antrum, which contains smooth muscle cells and ICC_{IM}, is that the membrane potential displays an ongoing discharge of membrane noise (19). When recordings are made from tissues devoid of ICC_{IM}, a discharge of membrane noise is not detected: these observations have been made on tissues obtained from wild type and W/W^V mutant mice (17, 20) or from Sl/Sl^d mice (43). When power spectral density curves are determined, those that contain ICC_{IM} have a characteristic shape, whereas those from the tissues lacking ICC_{IM} display power spectral density curves that reflect the properties of the recording system (19). When tissues are bathed in solutions containing agents such as BAPTA-AM or MAPTA-AM, which buffer the $[Ca^{2+}]_i$ to low levels, the discharge of membrane noise is reduced and discrete depolarizing potentials are detected. These potentials, which resemble the unitary potentials detected in ICC_{MY}, have reproducible time courses and have power spectral density curves with shapes identical to those obtained from control tissues (19). This indicates that individual ICC_{IM} spontaneously discharge unitary potentials which, when recordings are made from preparations containing many ICC_{IM}, are manifested as a discharge of membrane noise in control conditions. In isolated urethral ICC-like cells, a spontaneous discharge of unitary currents is also detected (51): these have similar time courses and amplitudes to those calculated to underlie antral unitary potentials (19, 51). Thus unitary potentials reflect the basic signaling mechanism used by ICC, with large membrane potential changes being generated when many unitary potentials occur synchronously. This contrasts with the way in which most excitable cells generate electrical activity, which relies on the activation of membrane-located voltage-dependent channels.

The discharge of membrane noise, as well as being inhibited by buffering $[Ca^{2+}]_i$, to low levels, is abolished by 2-APB, an agent that prevents the release of Ca^{2+} from IP_3-dependent stores (45). Moreover, as pointed out previously, all rhythmical electrical activity is absent in gastric tissues taken from mutant mice that lack IP_3 type-1 receptors (26). Together, these observations suggest that unitary potentials result from the release of Ca^{2+} from internal IP_3-dependent calcium stores and the subsequent activation of ion-selective channels. The nature of the membrane channels present in gastric ICC_{IM} is not understood. However, it has been shown that the discharge of membrane noise detected in antral preparations containing ICC_{IM} is blocked by a number of agents that block anion-selective channels (45), suggesting that it results from the opening of calcium-activated chloride channels. On the other hand, the discharge of membrane noise detected in preparations taken from mouse or guinea pig fundus is unaffected by a wide range of agents that block anion-selective channels (24). This indicates that the channels located in the mem-

branes of ICC_{IM} in different regions of the stomach have fundamentally different pharmacological properties: it may be that the ion selectivity of membrane channels in the ICC_{IM} varies with location.

In the gastric antrum, the frequency of unitary potentials is increased when ICC_{IM} are depolarized, thus giving rise to regenerative responses, i.e., the secondary component of the slow wave (Fig. 3A, ref. 19). The onset of the increase in frequency occurs with a minimum latency of about 1 s at 37°C (5, 19, 45), which suggests that depolarization activates a complex pathway, for example, increasing the production of a second messenger like IP_3 (5, 21 see also 52). Whatever the case, the observations indicate that a voltage sensing mechanism resides in the membranes of antral ICC_{IM} which, when activated, leads to a delayed increase in $[Ca^{2+}]_i$ and the subsequent discharge of unitary potentials (45).

In the fundus, which contains ICC_{IM} but fails to generate spontaneous activity, differences between the electrical properties of antral and fundal ICC_{IM} have recently been demonstrated (Fig. 3). In both mouse and guinea pig fundus preparations, ICC_{IM} appear to lack voltage sensitivity (Fig. 3B, ref. 24). Thus when depolarized, they failed to initiate regenerative response (Fig. 3B, ref. 24). Hence fundal ICC_{IM} are unable to coordinate their activity and they fail to generate spontaneous rhythmical activity. The simplest explanation for this finding is that fundal ICC_{IM} lack a voltage sensor.

As pointed out above, responses to excitatory cholinergic or inhibitory nitrergic nerve stimulation cannot be detected in antral and fundal tissues devoid of ICC_{IM}. This implies that the properties of ICC_{IM} are changed by neurally released ACh and NO. In the antrum, the responses to cholinergic nerve stimulation can be attributed to an increase in the rate of discharge of unitary potentials (32), suggesting that ACh may activate the same pathway as that activated by membrane depolarization. Conversely, in the antrum, where NO is the dominant inhibitory transmitter (49), neurally released NO slows the rate of discharge of unitary potentials, so giving rise to a moderate hyperpolarization (47). Furthermore, neurally released NO also prevents the increase in frequency of unitary potentials which is normally triggered by periods of membrane depolarization (Fig. 5, ref. 47).

In summary, ICC_{IM} generate an ongoing spontaneous discharge of unitary potentials. In the antrum, the frequency of unitary potentials is transiently increased either by periods of membrane depolarization or by neurally released ACh; conversely, their frequency is reduced by hyperpolarization and by neurally released NO. Studies on the properties of unitary potentials, generated by populations of antral ICC_{IM}, suggest that individual unitary potentials result from the release of Ca^{2+} from internal Ca^{2+} stores and the subsequent opening of calcium-activated, anion-selective channels in the antrum. Although unitary potentials are also generated spontaneously by fundal ICC_{IM}, in the fundus the frequency of generation of unitary potentials is not modulated by changes in membrane potential; nevertheless, a modulation of their frequency of discharge may well be involved in the processes of inhibitory and excitatory neuroeffector transmission.

Summary and conclusions

Studies on gastrointestinal ICC are in their infancy, only a few things are known and much remains to be learnt. In the antrum and the small intestine, ICC_{MY} generate a rhythmical discharge of pacemaker potentials that depolarize the adjacent longitudinal and circular muscle layers. The cellular mechanisms involved in the generation of pacemaker potentials are not fully understood. However, it is clear that the release of Ca^{2+} from internal IP_3-dependent stores plays a key part in their generation. In the circular layer of the antrum, ICC_{IM} demonstrate voltage sensitivity such that when the circular layer is depolarized, ICC_{IM} discharge unitary potentials at a high frequency: unitary potentials sum together to generate the secondary regenerative component of the slow wave. Whether the pacemaker depolarizations reaching the smooth muscle layers of the small intestine is augmented by ICC_{IM} is not known. The cellular mechanisms involved in generation of the secondary component of the antral slow wave are not fully understood, but again the release of Ca^{2+} from internal IP_3-dependent stores appears to be essential. In all regions of the stomach examined to date, ICC_{IM} have been found to be intermediatory cells in the pathway by which excitatory and inhibitory nerves modulate the excitability of the circular muscle layer. Thus when ICC_{IM} are absent, nerve stimulation is ineffective or severely attenuated. How inhibitory and excitatory influences are transferred to nearby smooth muscle cells is not known. A part of the transfer of information can be explained by simple electrical conduction, but this may only be part of the story, and it could be that ICC_{IM} are a source of second messengers that diffuse into nearby smooth muscle cells. In some regions of the gut, ICC_{IM} are normally present in very low numbers, for example, the longitudinal layer of the antrum. Presumably in these regions, neurally released transmitters can directly access smooth muscle cell membranes. Finally, it is clear that the properties of ICC vary with location in the gastrointestinal tract. As examples, pacemaker

potentials generated by ICC_{MY} in the antrum and small intestine occur at quite different frequencies and have quite different durations: the frequency of discharge of unitary potentials by antral ICC_{IM} is affected by changes in membrane potential, whereas that of fundal ICC_{IM} is not. An important direction for future research will be to understand how these differences occur and to discover whether the properties of ICC_{IM} vary in other regions of the gut.

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