Journal of Physiology (1991), **442**, *pp.* 31–45
With 12 figures
Printed in Great Britain

EFFECT OF VOLTAGE AND CYCLIC AMP ON FREQUENCY OF SLOW-WAVE-TYPE ACTION POTENTIALS IN CANINE COLON SMOOTH MUSCLE

BY JAN D. HUIZINGA, LAURA FARRAWAY AND ADRIAAN DEN HERTOG

From the Intestinal Disease Research Unit and Department of Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

(*Received 17 December 1990*)

SUMMARY

1. A non-L-type calcium conductance is involved in the generation of the initial part of the slow-wave-type action potential in colonic smooth muscle. The present study addresses the question whether this conductance is voltage or metabolically activated.

2. Current-induced hyperpolarization increased frequency and amplitude of slow waves measured in Krebs solution.

3. The upstroke potential was 'isolated' from the slow wave by superfusion with 'glucamine–nitrendipine' Krebs solution (NaCl was replaced by glucamine, nitrendipine was added).

4. Hyperpolarization up to −100 mV did not affect the upstroke potential frequency and increased its amplitude. Only hyperpolarization further than −100 mV decreased the frequency $\leqslant 20\%$, and reduced the amplitude $\leqslant 20\%$.

5. Depolarization did not affect the upstroke potential frequency.

6. Forskolin, but not 1,9-dideoxyforskolin dramatically decreased the upstroke potential frequency, without affecting other parameters including the resting membrane potential.

7. The effect of forskolin was mimicked by dibutyryl cyclic AMP, 8-bromo-cyclic AMP and 3-isobutyl-1-methylxanthine (IBMX), but not extracellular cyclic AMP.

8. The upstroke potential could not be evoked by depolarizing pulses after inhibition of activity by forskolin.

9. The effect of forskolin could be reversed by the calcium ionophore A23187.

10. In summary, voltage changes up to −40 mV and down to −100 mV do not, but changes in intracellular cyclic AMP do affect the frequency of the upstroke potential.

11. It is likely that intracellular metabolic activity, which may include cyclic AMP but not a voltage change, activates the conductance responsible for the generation of the upstroke potential.

MS 9009

INTRODUCTION

Recent electrophysiological and structural studies have revealed the cellular origin of the slow-wave-type action potentials, also commonly referred to as slow waves, in gastrointestinal smooth muscle. There is evidence in the lower oesophageal sphincter (Huizinga & Walton, 1989; Pintin-Quezada, Berezin, Daniel & Huizinga, 1990), small intestine (Hara, Kubota & Szurszewski, 1986; Suzuki, Prosser & Dahms, 1986) and colon (Smith, Reed & Sanders, 1987; Barajas-López & Huizinga, 1988; Berezin, Huizinga & Daniel, 1988) that the primary pacemaker is generated in an area containing a network of interstitial cells of Cajal (ICC). In the small intestine this area is located near the nerve plexus, between the circular and longitudinal muscle layer; in the colon it is located at the submucosal border of the circular muscle layer. Interstitial cells of Cajal may be the primary pacemaker cells, they do generate slow-wave-type action potentials as proven by combined electron microscopic (EM) and electrophysiological experiments (Barajas-López, Berezin, Daniel & Huizinga, 1989*a*). One of the interesting morphological features of interstitial cells of Cajal are the numerous mitochondria suggesting high intracellular metabolic activity. A link between metabolic activity and the generation of the action potentials has been suggested previously. One hypothesis has been that the action potentials were generated by sodium pump activity (Connor, Prosser & Weems, 1974), another hypothesis suggested a link between the Na^+–Ca^{2+} exchange mechanism and generation of slow waves (Tomita, 1981). One striking feature consistent with a metabolic association, is the temperature dependence of the *frequency*, but not the amplitude, of gastrointestinal action potential activity (El-Sharkawy & Daniel, 1975; Barajas-López, Chow, Den Hertog & Huizinga, 1989*b*). The above-described structural information and the data on origin and temperature sensitivity of the action potential frequency leads to the hypothesis that interstitial cells of Cajal may exhibit a conductance involved in the initiation of the action potentials that is regulated by intracellular metabolic activity.

In the preceding paper, evidence was presented that a non-L-type calcium conductance is involved in the initiation step of the slow-wave-type action potential. The present study addresses the question of whether a change in voltage or an intracellular (metabolic) event triggers this conductance change.

METHODS

Tissue preparation, recording of electrical activities and method of data analysis were described previously (Barajas-López & Huizinga, 1988; Huizinga, Farraway & Den Hertog, 1991).

The measurement of *I–V* curves has been described previously (Huizinga & Chow, 1988). The physical dimensions of the tissue were such that hyperpolarizing current reached all of the tissue, so that activities could not have been unaltered in some part of the tissue because of lack of sufficient hyperpolarization.

Solutions and drugs

Experiments were performed in pre-warmed (36·5–37·0 °C) Krebs solution and equilibrated with 95% O_2–5% CO_2; the tissue was continuously perfused with Krebs solution at a constant rate (1·5–3·5 ml min^{-1}). The composition of this solution was (mM): NaCl, 120·3; KCl, 5·9; $CaCl_2$, 2·5; $MgCl_2$, 1·2; $NaHCO_3$, 20·0; NaH_2PO_4, 1·2 and glucose, 11·5. The pH was 7·30–7·35.

After equilibration in Krebs solution, most experiments were performed in 'glucamine–nitrendipine' Krebs solution where NaCl was replaced by *N*-methyl-D-glucamine and nitrendipine (3×10^{-7} M) was added. The pH was adjusted with HCl. This procedure removes the plateau phase from the slow wave and also the contribution of L-type calcium channels to the upstroke potential, as described in Huizinga *et al.* (1991). Eighty per cent of the upstroke potential remains, however, and the frequency is not different from control activity. In this paper we refer to the activity remaining in 'glucamine–nitrendipine' Krebs solution as the upstroke potential (see Huizinga *et al.* 1991). The rate of rise of the slow-wave-type action potential was 187 ± 45 mV s^{-1}, and that of the 'upstroke potential' in 'glucamine–nitrendipine' Krebs solution, 144 ± 55 mV s^{-1}, not significantly different. Experiments designed to affect the initiating phase of the upstroke potential are best carried out under circumstances where the least possible conductances contribute to the upstroke potential.

RESULTS

Voltage sensitivity of the upstroke potential

In Krebs solution

The electrical activity observed in Krebs solution was as reported previously (Chow & Huizinga, 1987; Huizinga *et al.* 1991). In the current experiments, the control data for frequency and duration of the slow-wave-type action potentials were $5{\cdot}5 \pm 0{\cdot}1$ c.p.m. and $3{\cdot}9 \pm 0{\cdot}3$ s. The frequency of the slow-wave-type action potentials increased by hyperpolarization. In ten experiments where maximal hyperpolarization (i.e. leading to a stable membrane potential without distortion) was achieved 10–25 mV below resting membrane potential the frequency *increased* 6 ± 2 %, and the amplitude *increased* 18 ± 1 %. Concomitantly the slow-wave duration decreased 55 ± 4 %. In eight experiments maximal depolarization achieved (ranging from 10 to 20 mV) led to a reduction in frequency of 10 ± 3 %, a reduction in amplitude of 38 ± 3 %, with a concomitant increase in slow-wave duration of 90 ± 15 %.

In 'glucamine–nitrendipine' Krebs

Current-induced hyperpolarization was applied in an attempt to lower the membrane potential below threshold for voltage activation ($n = 10$). In none of the experiments was the upstroke potential inhibited. The upstroke potential duration was not affected, whereas at the most hyperpolarized potentials (< -100 mV) a small reduction in frequency ($\leqslant 20$ %) and amplitude ($\leqslant 20$ %) was sometimes observed (Fig. 1). Depolarizing pulses did not affect the duration or frequency of the upstroke potential.

Sensitivity of the upstroke potential to increased intracellular cyclic AMP

The following experiments on the upstroke potential were carried out in 'glucamine–nitrendipine' Krebs solution.

Effect of forskolin

The frequency of the upstroke potential was affected in a concentration-dependent manner by forskolin (Figs 2 and 3; Table 1). Forskolin (5×10^{-5} M) decreased the frequency 80–100 %. The upstroke amplitude was either not affected or slightly

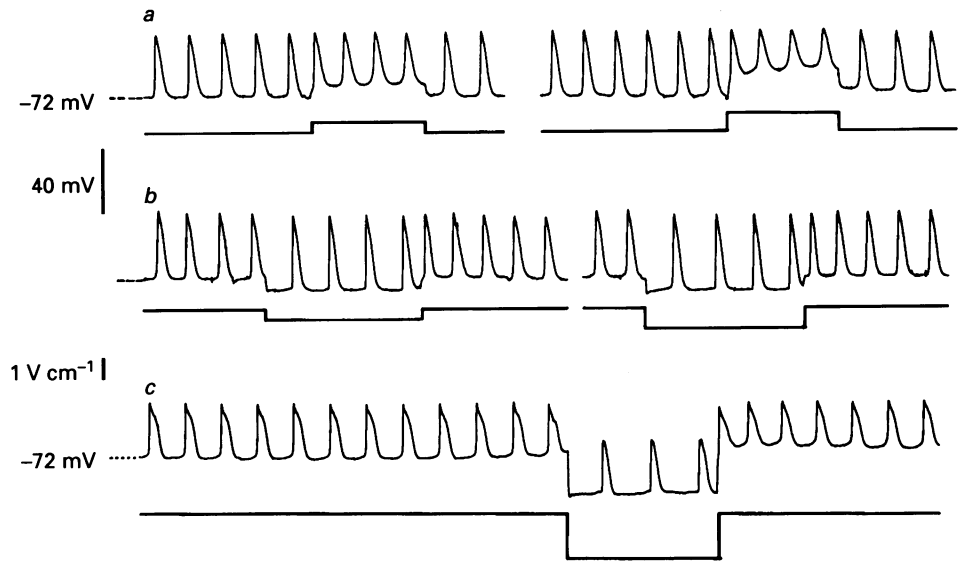

Fig. 1. Voltage sensitivity of the upstroke potential. Recordings were obtained in 'glucamine–nitrendipine' Krebs solution. Trace *a*, depolarization does not affect the frequency: the amplitude is restrained by a high-K^+ conductance at -50 mV (Huizinga *et al.* 1991). Trace *b*, hyperpolarization up to -100 mV does not affect the frequency whereas the amplitude increases, in that the maximum level of depolarization remains constant. Trace *c*, different experiment. Hyperpolarization below -100 mV never abolishes the upstroke potential, the amplitude does not decrease compared to control values but does sometimes compared to lower levels of hyperpolarization.

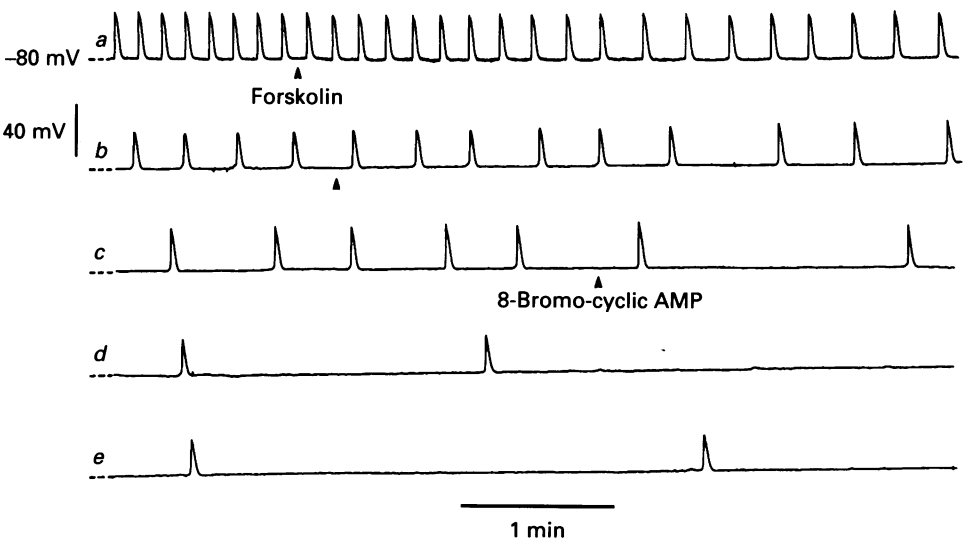

Fig. 2. Effect of forskolin and 8-bromo-cyclic AMP on the upstroke potential. Activity is recorded in 'glucamine–nitrendipine' Krebs solution. Shown is a continuous recording in one single cell. Dotted lines indicate control resting membrane potential at -80 mV. Trace *a*, control activity; at arrow forskolin (5×10^{-6} M) affects the upstroke potential frequency without an effect on any other parameter. Trace *b*, at arrow, the forskolin concentration is increased to 5×10^{-5} M. Trace *c*, at arrow, 2 mM-8-bromo-cyclic AMP is added reducing the frequency to a very low value.

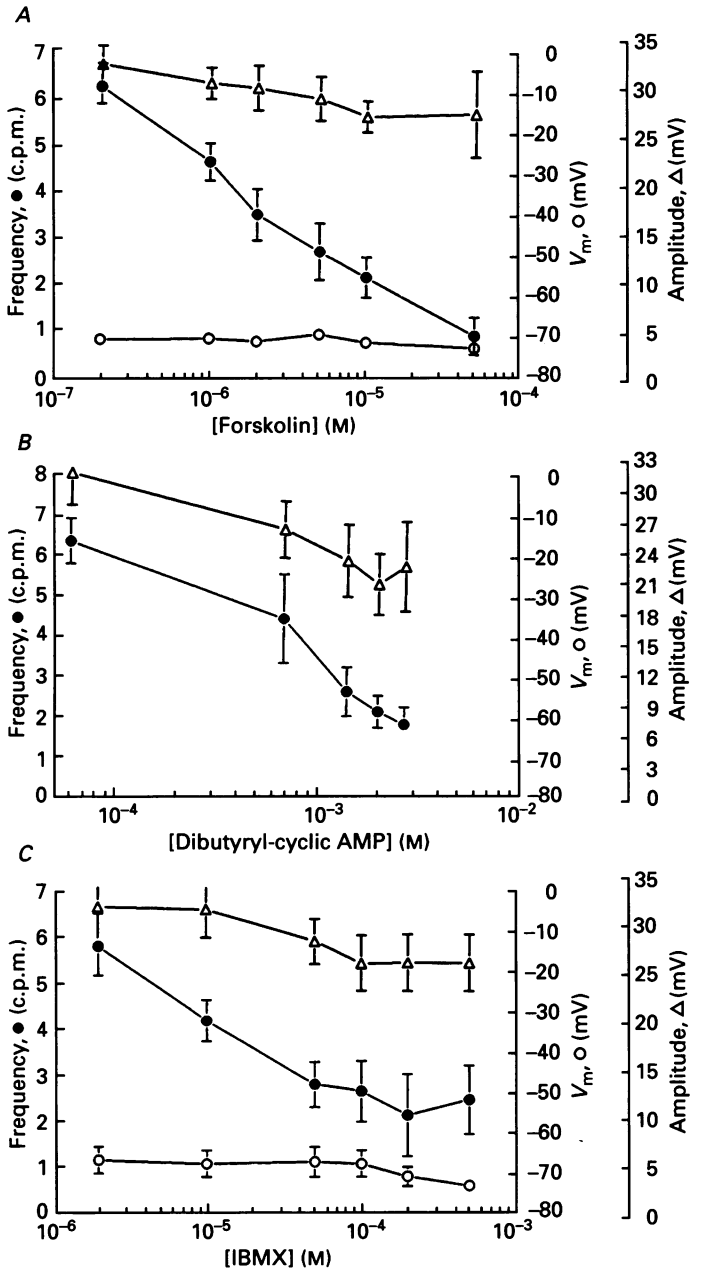

Fig. 3. Effect– concentration curves of forskolin (*A*), dibutyryl-cyclic AMP (*B*) and IBMX (*C*) on frequency and amplitude of the pacemaker potential and the resting membrane potential (V_m). Activities recorded in 'glucamine–nitrendipine' Krebs solution.

decreased but only at concentrations higher than 5×10^{-5} M. Forskolin did not affect the resting membrane potential (Fig. 3) nor the duration and rate of rise of the upstroke potential (Table 1).

Forskolin and input resistance

The inhibition of the upstroke potential frequency occurred without any change in input resistance. Figure 4 shows typical steady-state *I–V* curves in the absence and presence of forskolin. The slopes in the hyperpolarizing quadrant were $33{\cdot}9 \pm 3.0$ mV s^{-1} in control conditions and $28{\cdot}7 \pm 4{\cdot}8$ mV s^{-1} in the presence of

TABLE 1. Effects of forskolin, dibutyryl-cyclic AMP and IBMX on characteristics of upstroke potential

	Duration (s)	Rate of rise (mV s^{-1})	Slope of *I–V* curve
Forskolin			
Control	$1{\cdot}8 \pm 0{\cdot}15$ (9)	$118{\cdot}9 \pm 37{\cdot}6$ (8)	$31{\cdot}2 \pm 5{\cdot}2$ (9)
10^{-6} M	$1{\cdot}6 \pm 0{\cdot}18$ (6)	$119{\cdot}5 \pm 55{\cdot}7$ (5)	$27{\cdot}9 \pm 4{\cdot}2$ (5)
2×10^{-6} M	$1{\cdot}7 \pm 0{\cdot}27$ (4)	$127{\cdot}8 \pm 83{\cdot}7$ (3)	$29{\cdot}5 \pm 6{\cdot}3$ (3)
5×10^{-6} M	$1{\cdot}8 \pm 0{\cdot}25$ (4)	$141{\cdot}7 \pm 95{\cdot}8$ (3)	$26{\cdot}4 \pm 3{\cdot}4$ (5)
10^{-5} M	$1{\cdot}7 \pm 0{\cdot}17$ (7)	$165{\cdot}2 \pm 72{\cdot}3$ (4)	$25{\cdot}3 \pm 5{\cdot}0$ (6)
5×10^{-5} M	$2{\cdot}2 \pm 0{\cdot}27$ (3)	34·2 (1)	$26{\cdot}8 \pm 3{\cdot}6$ (2)
Dibutyryl-cyclic AMP			
Control	$2{\cdot}0 \pm 0{\cdot}16$ (5)	$85{\cdot}6 \pm 10{\cdot}8$ (2)	
$6{\cdot}8 \times 10^{-4}$ M	$1{\cdot}9 \pm 0{\cdot}12$ (4)	$61{\cdot}4 \pm 10{\cdot}0$ (2)*	
$1{\cdot}4 \times 10^{-3}$ M	$1{\cdot}7 \pm 0{\cdot}20$ (5)*	$74{\cdot}8 \pm 12{\cdot}3$ (2)*	
2×10^{-3} M	$1{\cdot}8 \pm 0{\cdot}25$ (4)	67·6 (1)	
$2{\cdot}7 \times 10^{-3}$ M	$1{\cdot}8 \pm 0{\cdot}25$ (4)	83·3 (1)	
IBMX			
Control	$2{\cdot}1 \pm 0{\cdot}1$ (5)	$102{\cdot}3 \pm 29{\cdot}3$ (5)	
10^{-5} M	$1{\cdot}8 \pm 0{\cdot}12$ (4)	$97{\cdot}6 \pm 22{\cdot}4$ (4)	
5×10^{-5} M	$1{\cdot}6 \pm 0{\cdot}07$ (3)*	$121{\cdot}4 \pm 21{\cdot}4$ (2)	
10^{-4} M	$1{\cdot}6 \pm 0{\cdot}1$ (2)	$66{\cdot}2 \pm 32{\cdot}0$ (2)	
2×10^{-4} M	$1{\cdot}7 \pm 0{\cdot}15$ (3)*	$70{\cdot}4 \pm 2{\cdot}8$ (2)	
5×10^{-4} M	$1{\cdot}9 \pm 0{\cdot}31$ (3)	$61{\cdot}5 \pm 19{\cdot}1$ (2)	

* $P < 0{\cdot}05$ compared to control.
Values are means ± S.E.M. with number of experiments in parentheses.

10^{-5} M-forskolin ($n = 5$), not significantly different. The slopes were also calculated in the presence of 10^{-6}, 2×10^{-6}, 5×10^{-6} and 5×10^{-5} M-forskolin and found not to be different from control.

1, 9-Dideoxyforskolin

To obtain evidence that the effect of forskolin was due to activation of adenylate cyclase and not to non-specific effects (Seamon, Vaillancourt, Edwards & Daly, 1984), the effect of 1,9-dideoxyforskolin (5×10^{-5} M) was studied. This compound had no effect on any parameters of the electrical activity ($n = 3$, Fig. 5). 1,9-Dideoxyforskolin was obtained from two different batches.

Forskolin in Krebs solution

To ascertain that the effect of forskolin was not peculiar to the 'glucamine–nitrendipine' solution, the effect of forskolin was studied in normal Krebs solution. Forskolin (10^{-5} M) abolished the plateau potential, thereafter a gradual decline in upstroke potential frequency was observed, similar to that observed in

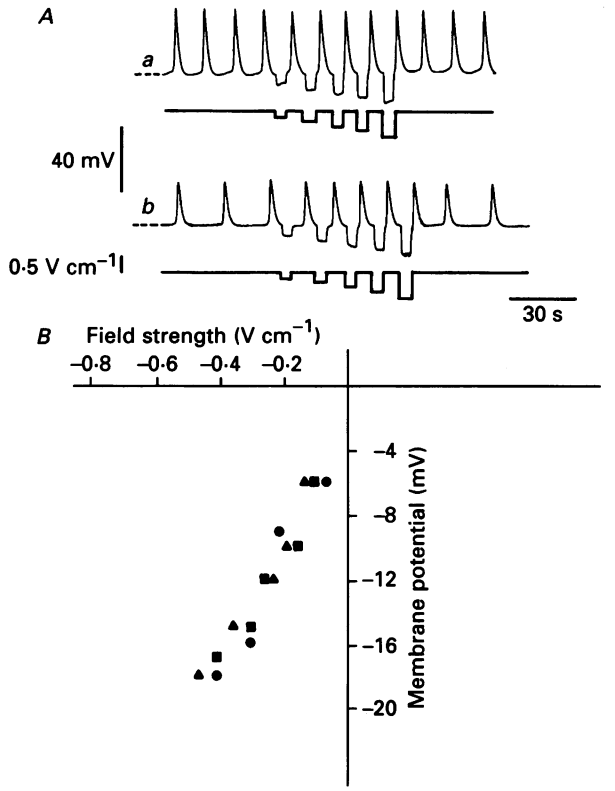

Fig. 4. Forskolin does not affect the input resistance. *A*: *a*, electronic potentials evoked in 'glucamine–nitrendipine' Krebs solution, *b*, electrotonic potentials evoked in the presence of forskolin (10^{-5} M). *B*, current–voltage relationship in control (●) and in the presence of two different forskolin concentrations, 10^{-6} M (■) and 10^{-5} M (▲). The slopes of the linear parts of the *I–V* curves were not significantly different.

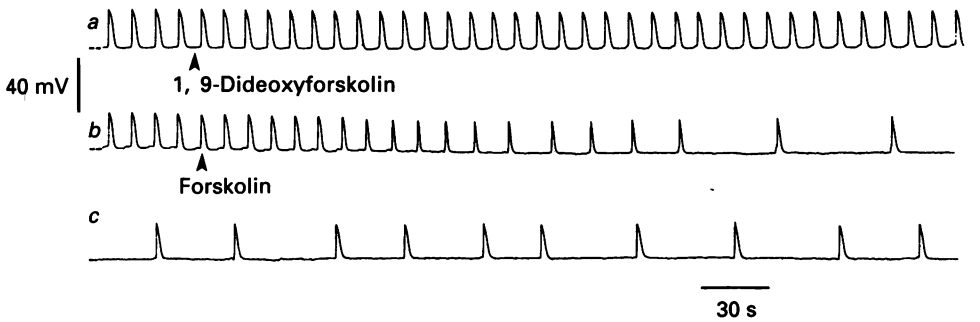

Fig. 5. Comparison of effects of 1,9-dideoxyforskolin and forskolin. Trace *a*, 1,9-dideoxyforskolin (10^{-5} M), applied at arrow, has no effect on electrical activity. Traces *b* and *c* forskolin (10^{-5} M), on the same cell, has its characteristic effect. The resting membrane potential (dashed line) was −78 mV.

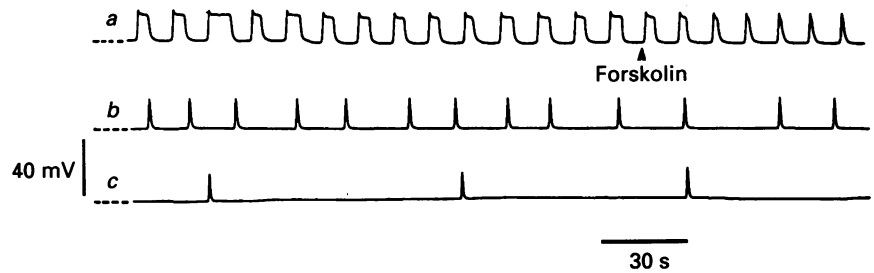

Fig. 6. Effects of forskolin on slow-wave-type action potentials in Krebs solution. Dashed line represents resting membrane potential at −60 mV. At arrow, forskolin (10^{-5} M) is added. Tracings represent a continuous recording.

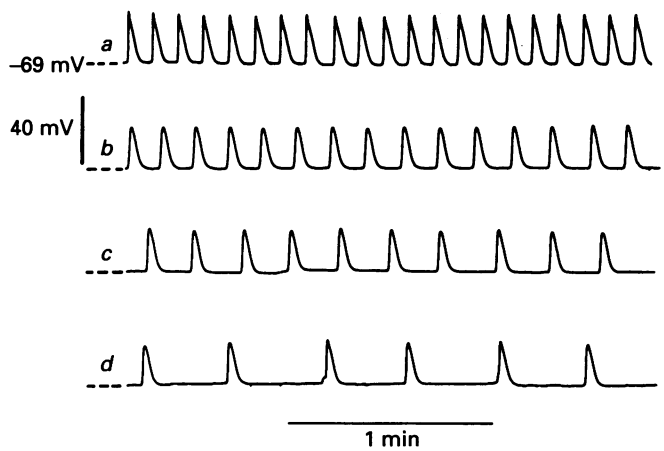

Fig. 7. Effects of dibutyryl-cyclic AMP. Dibutyryl-cyclic AMP induced a concentration-dependent reduction in the frequency of the pacemaker potential. The dashed lines show the control resting membrane potential of −69 mV. Trace *a*, activity in 'glucamine–nitrendipine' Krebs solution. Traces *b*, *c*, and *d* are portions of a continuous recording from one cell 2 min after addition of dibutyryl-cyclic AMP at concentrations of 0·5 mM (*b*), 1 mM (*c*), 3 mM (*d*). Note that the concentrations indicate what is given extracellularly, the true intracellular concentration is not known.

'glucamine–nitrendipine' Krebs solution (Fig. 6). Forskolin reduced the slow-wave frequency from 6·7±1·3 to 2·9±0·6 c.p.m., and the slow-wave amplitude from 34·4±0·9 to 31·6±0·8 mV ($n = 6$). No effect on the membrane potential was observed.

Dibutyryl cyclic AMP

Dibutyryl cyclic AMP affected the upstroke potential in a manner very similar to forskolin (Figs 3 and 7; Table 1). The reduction in frequency was pronounced whereas no effect on membrane potential was observed. The amplitude of the upstroke potential was also not affected. The duration was slightly decreased (from 2·0 to 1·8 s; Table 1). Dibutyryl cyclic AMP did not affect the input resistance.

After forskolin or dibutyryl cyclic AMP reduced the frequency of upstroke potential to zero, long-lasting depolarizing pulses (1 min) or hyperpolarizing pulses

did not restore activity. Brief depolarizing pulses did not evoke upstroke potentials either (Fig. 8). When upstroke potential activity was reduced to between 4 and 2 c.p.m. by forskolin, depolarizing pulses could evoke some activity in some experiments but the original frequency could not be restored.

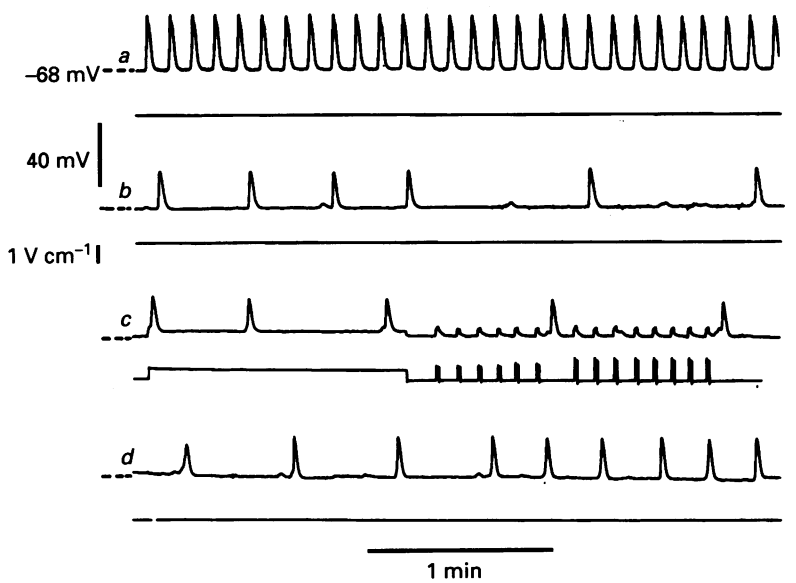

Fig. 8. In the presence of 2 mM-dibutyryl-cyclic AMP, depolarizing pulses do not evoke upstroke potentials. Trace *a*, activity in 'glucamine–nitrendipine' Krebs solution. Trace *b*, activity in the presence of dibutyryl-cyclic AMP. Trace *c*, depolarizing current pulses evoke electrotonic potentials, but upstroke potentials are not initiated. Trace *d*, recovery in 'glucamine–nitrendipine' Krebs solution.

8-Bromo-cyclic AMP

8-Bromo-cyclic AMP affected the upstroke potential in a way very similar to that of dibutyryl-cyclic AMP and forskolin ($n = 4$; Fig. 2). The effects of forskolin and 8-bromo-cyclic AMP were additive. Under those conditions where 5×10^{-5} M-forskolin did not reduce the frequency to zero, addition of 8-bromo-cyclic AMP did reduce the frequency further (Fig. 2). Addition of 3 mM-8-bromo-cyclic AMP to 5×10^{-5} M-forskolin reduced the frequency from $1{\cdot}5 \pm 0{\cdot}4$ to $0{\cdot}3 \pm 0{\cdot}2$ c.p.m. ($n = 3$).

As a control the effect of cyclic AMP was studied. Cyclic AMP up to 3 mM did not have any effect on the upstroke potential ($n = 3$) indicating that effects with 8-bromo-cyclic AMP and dibutyryl-cyclic AMP were not due to action on extracellular receptors.

IBMX

IBMX is a non-specific phosphodiesterase inhibitor that will cause increase in both intracellular cyclic AMP and cyclic GMP. IBMX reduced the upstroke potential frequency in a concentration-dependent manner (Figs 3 and 9). The other parameters of the upstroke potential were not or marginally affected (Table 1).

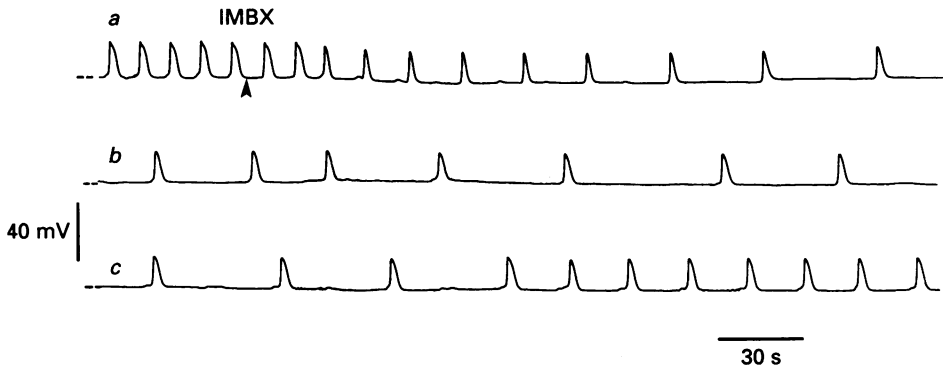

Fig. 9. The effect of IBMX. In trace *a* at arrow, IBMX (10^{-4} M) is given resulting in reduction in upstroke potential frequency. In trace *c* IBMX is washed out resulting in recovery of the upstroke frequency.

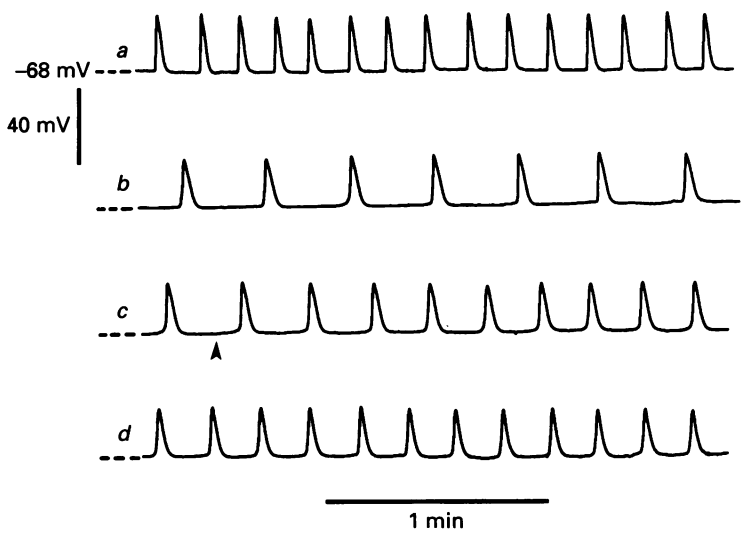

Fig. 10. A23187 reverses the inhibitory effect of dibutyryl-cyclic AMP on upstroke potential frequency. Trace *a*, activity in 'glucamine–nitrendipine' Krebs solution. Trace *b*, in the presence of 1 mM-dibutyryl-cyclic AMP. Trace *c*, at arrow, A23187 (10^{-5} M) is added which increases the upstroke potential frequency.

Cyclic AMP and calcium

Since decrease in extracellular calcium can reduce the upstroke potential frequency (Huizinga *et al.* 1991), the possibility exists that increase in intracellular calcium may have effects opposite to that of intracellular cyclic AMP. Indeed, the calcium ionophore A23187 (10^{-7}–10^{-5} M) reversed the effects of dibutyryl-cyclic AMP on the upstroke potential frequency (Fig. 10). In four experiments where dibutyryl-cyclic AMP reduced the frequency from $6{\cdot}1 \pm 0{\cdot}8$ to $2{\cdot}4 \pm 0{\cdot}5$, A23187 restored the frequency to $4{\cdot}5 \pm 1{\cdot}1$ c.p.m.

A23187, at higher concentrations, affected in addition to the frequency, also the

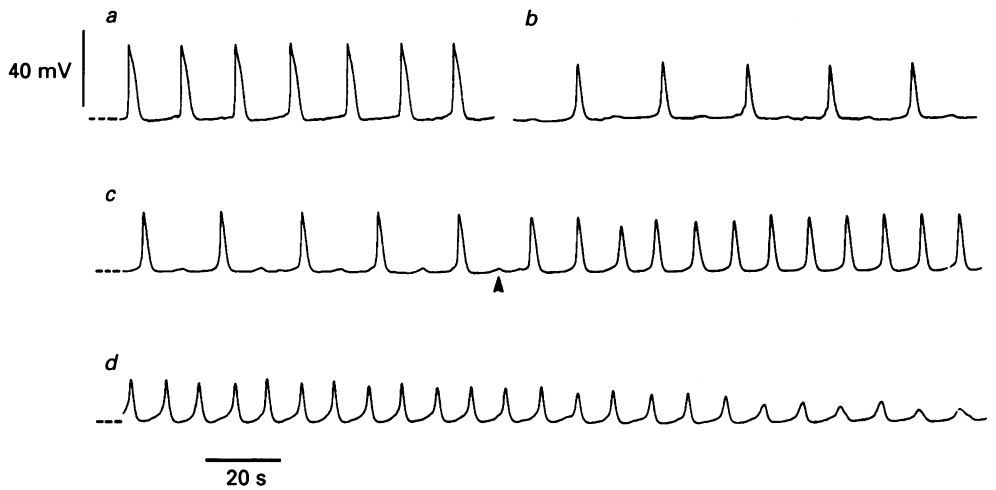

Fig. 11. A23187 affects upstroke potential frequency and, at higher concentrations, upstroke potential amplitude. Trace *a*, activity in 'glucamine–nitrendipine' Krebs solution. Trace *b*, in the presence of dibutyryl-cyclic AMP (2×10^{-3} M) the upstroke potential frequency decreased and in trace *c* A23187 (5×10^{-6}M) was added at arrow, which increased the frequency to a value higher than the control value. Trace *d*, 10 min later in the presence of A23187 (10^{-5} M) the upstroke potential amplitude gradually decreased. The resting membrane potential remained at −75 mV (dashed lines).

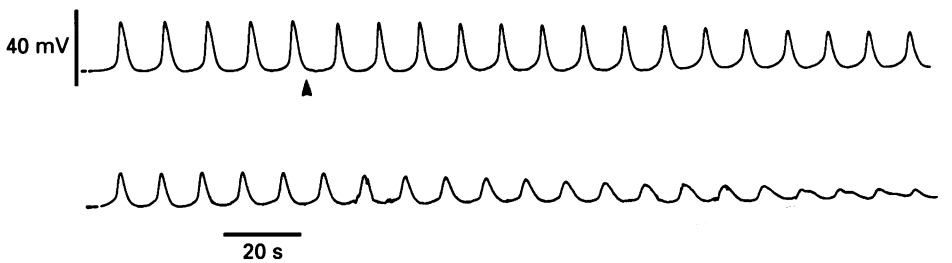

Fig. 12. Effect of A23187 in 'glucamine–nitrendipine' Krebs solution. Continuous recording. At arrow, A23187 (10^{-6} M) caused the upstroke potential amplitude to decrease. The dashed lines represent the resting membrane potential at −71 mV.

amplitude of the upstroke potential. In five experiments, in the presence of dibutyryl-cyclic AMP (2×10^{-3} M; $n = 4$) or forskolin (10^{-5} M; $n = 1$), the amplitude decreased gradually to zero after addition of 5×10^{-6} to 10^{-5} M-A23187 (Fig. 11) In control 'glucamine–nitrendipine' Krebs solution, in which the frequency is relatively high (Fig. 3), A23187 decreased the amplitude without affecting the frequency (Fig. 12).

DISCUSSION

The present study provides supporting evidence for the hypothesis that the upstroke potential is not initiated by a voltage change. First, after 'isolating' the initial part of the upstroke potential by 'glucamine–nitrendipine' Krebs solution, it

was observed that there was no slowly developing depolarization preceding the upstroke potential. An argument could be made that no recordings were obtained from true pacemaker cells. However, recordings were obtained from the most superficial cells at the submucosal surface where the network of ICC is situated (Barajas-López *et al.* 1989*a*). Secondly, current-induced hyperpolarization up to −110 mV did not abolish the upstroke potential. Whereas hyperpolarization in 'glucamine–nitrendipine' Krebs solution below −85 mV reduced the upstroke potential frequency in some experiments, hyperpolarization in Krebs solution actually increased the slow-wave frequency. Current-induced depolarization did not affect the upstroke potential frequency. Previous studies (Barajas-López, Den Hertog & Huizinga, 1989*c*) have shown that the slow wave can be generated at membrane potentials up to −40 mV. Thus, over a range of −110 to −40 mV slow waves are generated at virtually identical frequency. The mechanism that gives the cell its rhythmicity is therefore rather voltage insensitive. Thirdly, a dramatic decrease in the frequency of the upstroke potential by increase in intracellular cyclic AMP was not accompanied by any change in resting membrane potential nor was it accompanied by any change in input resistance.

The results from the present study on colonic tissue are consistent with several reports from other gastrointestinal tissues. Connor in 1979 noted '...there is a conspicuous lack of slow depolarization leading into the slow wave upstroke...' referring to the stomach and small intestine. It appears that also in these tissues, the upstroke potential starts sharply from a stable resting membrane potential. This could occur when the microelectrode only occasionally penetrates a true pacemaker cell, which seems unlikely, since Suzuki *et al.* (1986), in the cat intestine, made numerous penetrations in cells in the area where pacemaker activity originates, the ICC network in the myenteric plexus region. The lack of voltage sensitivity of the slow wave was also noted in the guinea-pig stomach (Tomita, 1981). Hyperpolarization of this tissue had no effect on the initial part of the upstroke potential.

The present study shows that a change in concentration of intracellular cyclic AMP affects the frequency of the upstroke potential and hence that of the slow-wave-type action potential. This effect is not an indirect effect of a change in amplitude or membrane potential, noteworthy since in most models of excitability, the frequency is a function of the oscillation amplitude (Connor, 1979). Independent frequency modulation is consistent with the model developed by Bardakjian (Bardakjian & Bot, 1987; Bardakjian & Lau, 1990). The present study shows that the upstroke potential frequency can be affected independently of changes in upstroke potential amplitude. The marked effect of cyclic AMP on upstroke potential frequency, together with the lack of voltage sensitivity of the slow waves, suggests that cyclic nucleotides may be an important component of the 'clock' or the mechanism that gives the cell its rhythmicity. The intracellular cyclic AMP concentration (and other components involved) will be determined by the relative activity of synthesis and break-down, and hence will be metabolically sensitive. Strong support for a metabolically sensitive clock comes from the observation that the slow-wave frequency is very sensitive to temperature. In fact, the slow-wave frequency is far more sensitive to a decrease in temperature than the slow-wave amplitude (Barajas-López *et al.* 1989*a*). This has also been observed in the small intestine (El-Sharkawy

& Daniel, 1975; Dahms, Prosser & Suzuki, 1987) and stomach (Magaribuchi, Ohbu, Sakamoto & Yamamoto, 1972).

Other than cyclic AMP, changes in extracellular (and, probably, consequently intracellular) calcium also affect the slow-wave frequency (preceding paper, Huizinga *et al.* 1991). In the small intestine (Connor, 1979), and the stomach (Magaribuchi *et al.* 1972) a gradual decline in slow-wave frequency is observed by removal of extracellular calcium. We observed that a calcium ionophore can reverse the decline in upstroke potential frequency induced by forskolin. It suggests the possibility that calcium and cyclic AMP may work antagonistically in the generation of the clock mechanism.

Metabolic regulation of slow-wave activity has been proposed previously. The most discussed hypothesis has been that cyclic activity of an electrogenic sodium pump is generating the slow wave (Connor *et al.* 1974). Recently (Dahms *et al.* 1987), this hypothesis was affirmed for the small intestine based on the observation that ouabain abolishes slow waves. However, in the canine colon, it has been established that ouabain abolishes slow waves by depolarization and that current-induced hyperpolarization restores slow-wave activity in the presence of ouabain (Barajas-López *et al.* 1989*b*). Tomita (1981) suggested that a Na^+–Ca^{2+} exchange mechanism may be responsible, but this is unlikely because of the lack of sensitivity of the slow waves to removal of extracellular sodium (Barajas-López *et al.* 1989*c*). In the light of the present findings and those of the preceding paper we put forward the hypothesis that the slow-wave-type action potential is initiated by metabolic regulation of a non-L-type calcium conductance. The precise nature of the metabolic 'clock' needs further investigation but cyclic AMP and calcium may be important. There is evidence for inhibition of ion channel conductance by increase in intracellular cyclic AMP in neurons (Siegelbaum, Camardo & Kandel, 1982) and regulation of calcium channel activity by cyclic nucleotides in cardiac tissue (Sperelakis, 1988), but such a direct link in colonic muscle cells remains speculative.

A problem perceived with metabolic regulation of slow-wave activity is that it may not provide a mechanism for phase locking and synchronization. It is likely, however, that the metabolic regulation of slow-wave activity takes place in the ICC network, that the generated pacemaker activity is transmitted to the smooth muscle cells which take active part in generation of the slow waves (Liu, Daniel & Huizinga, 1990) and subsequently the slow waves are actively propagated through the muscle layer. Active propagation was shown with simultaneous recording of slow waves at different sites in the circular muscle of the canine stomach (Bauer & Sanders, 1985). Thus metabolic activation takes place in the network of ICC, densely coupled through gap junctions and rich in mitochondria. Phase locking and synchronization in the muscle layer takes place because of electrotonic coupling.

We have appreciated many discussions on cyclic nucleotide metabolism with Dr Donald H. Maurice. We thank Dr Paul Sabourin for performing the experiment which led to Fig. 6. Financial support came from the Medical Research Council of Canada. Jan D. Huizinga holds an MRC scholarship.

REFERENCES

BARAJAS-LÓPEZ, C., BEREZIN, I., DANIEL, E. E. & HUIZINGA, J. D. (1989*a*). Pacemaker activity recorded in interstitial cells of Cajal of the gastrointestinal tract. *American Journal of Physiology* **257**, C830–835.

BARAJAS-LÓPEZ, C., CHOW, E., DEN HERTOG, A. & HUIZINGA, J. D. (1989*b*). Role of the sodium pump in pacemaker generation in colonic smooth muscle. *Journal of Physiology* **416**, 369–383.

BARAJAS-LÓPEZ, C., DEN HERTOG, A. & HUIZINGA, J. D. (1989*c*). Ionic basis of pacemaker generation in colonic smooth muscle. *Journal of Physiology* **416**, 385–402.

BARAJAS-LÓPEZ, C. & HUIZINGA, J. D. (1988). Heterogeneity in spontaneous and tetraethylammonium induced intracellular electrical activity in colonic circular muscle. *Pflügers Archiv* **412**, 203–210.

BARDAKJIAN, B. L. & BOT, S. D. (1987). Mapped clock oscillators: Their use to model gastric ECA. *Digestive Diseases and Sciences* **32**, 902 (abstract).

BARDAKJIAN, B. L. & LAU, M. M. (1990). The refractory properties of mapped clock oscillators representing smooth muscle electrical oscillations. *Progress in Clinical and Biological Research* **327**, 627–634.

BAUER, A. J. & SANDERS, K. M. (1985). Gradient in excitation–contraction coupling in canine gastric antral circular muscle. *Journal of Physiology* **369**, 283–294.

BEREZIN, I., HUIZINGA, J. D. & DANIEL, E. E. (1988). Interstitial cells of Cajal in the canine colon: A special communication network at the inner border of the circular muscle. *Journal of Comparative Neurology* **273**, 42–51.

CHOW, E. & HUIZINGA, J. D. (1987). Myogenic electrical control activity in longitudinal muscle of human and dog colon. *Journal of Physiology* **392**, 21–34.

CONNOR, J. A. (1979). On exploring the basis for slow potential oscillations in the mammalian stomach and intestine. *Journal of Experimental Biology* **81**, 153–173.

CONNOR, J. A., PROSSER, C. L. & WEEMS, W. A. (1974). A study of pace-maker activity in intestinal smooth muscle. *Journal of Physiology* **240**, 671–701.

DAHMS, V., PROSSER, C. L. & SUZUKI, N. (1987). Two types of 'slow waves' in intestinal smooth muscle. *Journal of Physiology* **392**, 51–69.

EL-SHARKAWY, T. Y. & DANIEL, E. E. (1975). Electrical activity of small intestinal smooth muscle and its temperature dependence. *American Journal of Physiology* **229** (5), 1268–1276.

HARA, Y., KUBOTA, M. & SZURSZEWSKI, J. H. (1986). Electrophysiology of smooth muscle of small intestine of some mammals. *Journal of Physiology* **372**, 501–520.

HUIZINGA, J. D. & CHOW, E. (1988). Electrotonic current spread in colonic muscle. *American Journal of Physiology* **254**, G702–710.

HUIZINGA, J. D., FARRAWAY, L. & DEN HERTOG, A. (1991). Generation of slow-wave-type action potentials in canine colon smooth muscle involves a non-L-type Ca^{2+} conductance. *Journal of Physiology* **442**, 15–29.

HUIZINGA, J. D. & WALTON, P. (1989). Pacemaker activity in the proximal lower oesophageal sphincter of the dog. *Journal of Physiology* **408**, 19–30.

LIU, L. W. C., DANIEL, E. E. & HUIZINGA, J. D. (1990). Colonic circular muscle without the network of interstitial cells of Cajal can generate slow waves through different mechanisms. *Journal of Gastrointestinal Motility* (in the Press).

MAGARIBUCHI, T., OHBU, T., SAKAMOTO, Y. & YAMAMOTO, Y. (1972). Some electrical properties of the slow potential changes recorded from the guinea pig stomach in relation to drug actions. *Japanese Journal of Physiology* **22**, 333–352.

PINTIN-QUEZADA, J., BEREZIN, I., DANIEL, E. E. & HUIZINGA, J. D. (1990). Bundles of smooth muscle, interstitial cells of Cajal and nerves intertwine with skeletal muscle and generate pacemaker activity in canine esophagus. *Journal of Gastrointestinal Motility* (in the Press).

SEAMON, K. B., VAILLANCOURT, R., EDWARDS, M. & DALY, J. W. (1984). Binding of [^{3}H]forskolin to rat brain membranes. *Proceedings of the National Academy of Sciences of the USA* **81**, 5081–5085.

SIEGELBAUM, S. A., CAMARDO, J. S. & KANDEL, E. R. (1982). Serotonin and cyclic AMP close single K^+ channels in *Aplysia* sensory neurones. *Nature* **299**, 413–417.

SMITH, T. K., REED, J. B. & SANDERS, K. M. (1987). Origin and propagation of electrical slow waves in circular muscle of canine proximal colon. *American Journal of Physiology* **252**, C215–224.

SPERELAKIS, N. (1988). Regulation of calcium slow channels of cardiac muscle by cyclic nucleotides and phosphorylation. *Journal of Molecular and Cellular Cardiology* **20**, suppl. II, 75–105.

SUZUKI, N., PROSSER, C. L. & DAHMS, V. (1986). Boundary cells between longitudinal and circular layers: essential for electrical slow waves in cat intestine. *American Journal of Physiology* **250**, G287–294.

TOMITA, T. (1981). Electrical activity (spikes and slow waves) in gastrointestinal smooth muscles. In *Smooth Muscle*, ed. BÜLBRING, E., pp. 127–156. Arnold, London.