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INHIBITION OF CALCIUM-DEPENDENT SPIKE AFTER-HYPERPOLARIZATION INCREASES EXCITABILITY OF RABBIT VISCERAL SENSORY NEURONES

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SUMMARY

1. Conventional intracellular recordings were made from rabbit nodose neurones *in vitro*. Prostagladins D_2 and E_2, but not $F_{2\alpha}$, produced a selective, concentration-dependent (1–100 nM) inhibition of a slow, Ca^{2+}-dependent spike after-hyperpolarization (a.h.p.). Block of the slow a.h.p. was accompanied by an increased membrane resistance and a small (< 10 mV) depolarization of the membrane potential. Inhibition of the slow a.h.p. produced no change in the voltage–current relationship other than the increased membrane resistance.

2. In C neurones with slow a.h.p.s, trains of brief depolarizing current pulses (2 ms duration, 0·1–10 Hz) could not elicit repetitive action potentials without failure at rates above 0·1 Hz. By contrast, C neurones without slow a.h.p.s could respond at stimulus frequencies up to 10 Hz. The frequency-dependent spike firing ability of slow a.h.p. neurones was eliminated by inhibition of the slow a.h.p.

3. Action potentials were also evoked by intrasomatic injection of paired, depolarizing current ramps (1 nA/10 ms, 0·1–5 s inter-ramp interval). For neurones without a slow a.h.p., the current threshold and number of evoked spikes were the same for both ramps, and the ramps were nearly superimposable. In neurones with a slow a.h.p., the current threshold for the first spike in the second ramp was greatly increased (300–500%) and the number of evoked spikes was reduced. Following inhibition of the slow a.h.p., the current threshold and number of evoked spikes was the same for both ramps.

4. Forskolin, a direct activator of the catalytic subunit of adenylate cyclase, also produced a concentration-dependent inhibition of the slow a.h.p., with 50% block at 30 nM. Prostaglandin D_2 and forskolin produced identical enhancement of excitability in C neurones and neither substance produced any effect on C neurones that could not be attributed to inhibition of the Ca^{2+}-dependent K^+ conductance associated with the slow a.h.p. We propose that, in some visceral sensory neurones, the level of excitability is regulated by cyclic AMP-mediated control of the slow a.h.p.

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INTRODUCTION

Ca^{2+}-dependent K^+ conductances ($g_{K(Ca)}$) are important regulators of membrane excitability in several neuronal populations because they contribute to spike repolarization and to the control of repetitive spike firing (Baldissera & Gustafsson, 1974*a*, *b*; Meech, 1978; MacDermott & Weight, 1982; Madison & Nicoll, 1984). The Ca^{2+}-dependent K^+ conductances represent a very heterogeneous class of K^+ conductances. They all decrease excitability by increasing membrane conductance and hyperpolarizing the membrane potential, but differ markedly in their temporal characteristics.

A subpopulation of neurones associated with unmyelinated, slowly conducting fibres (C neurones) located in the rabbit nodose ganglion possess a slow, Ca^{2+}-dependent spike after-hyperpolarization (a.h.p.) (Fowler, Greene & Weinreich, 1985*a*; see also Higashi, Morita & North, 1984). After one or more spikes, the slow a.h.p. exhibits a particularly long duration (2–30 s), a delayed onset (20–80 ms) and a slow rise time (τ_{on} 200–500 ms). These characteristics restrict the spike firing ability of these C neurones to a brief burst of spikes followed by a long refractory period (our unpublished observation). In previous reports we showed that prostaglandins selectively blocked the slow a.h.p. (Fowler *et al.* 1985*a*; Fowler, Wonderlin & Weinreich, 1985*b*). Here we have examined further the block of the slow a.h.p. by prostaglandins, and demonstrated that inhibition of the slow a.h.p. increases the excitability of these neurones by reducing the post-spike refractory period. Our results provide a model for the cellular basis of the sensitization, *in vivo*, of some visceral sensory C neurones by autocoids such as the prostaglandins.

METHODS

Nodose ganglia from sodium pentobarbitone-anaesthetized New Zealand rabbits (2–2·5 kg) were prepared as described previously (Fowler *et al.* 1985*a*), except that the ganglia were incubated with 0·1 % collagenase (Sigma type IV; 60 min at 25 °C or 30 min at 37 °C) to loosen the intraganglionic connective tissue. The slow a.h.p. was not affected by collagenase treatment. Each ganglion was sliced in half with a razor chip and the hemi-ganglia were pinned out on a nylon mesh grid (125 μm openings) in a recording chamber. Perfusate flowed over the tissue and down through the mesh (flow rate 1–2·5 ml/min) into a collecting chamber where it was aspirated with a suction pipette. With this chamber the perfusate level could be lowered to within 50 μm above the ganglion surface, which minimized stray capacitance between the microelectrode and the bath and enhanced the discontinuous current-injection procedure (see below).

The composition of the Locke (perfusate) solution was (mM): NaCl, 136; KCl, 5·6; $MgCl_2$, 1·2; $CaCl_2$, 2·2; NaH_2PO_4, 1·2; $NaHCO_3$, 14·3; and dextrose, 11. The Locke solution was equilibrated with 95 % O_2/5 % CO_2 (pH 7·2–7·4) and warmed to 29–30 °C with a Peltier element located adjacent to the recording chamber. The perfusate temperature 1 cm up-stream from the ganglion was continuously monitored with a microthermistor.

Stock solutions of prostaglandins D_2 (PGD_2), E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) (Sigma, St Louis, MO, U.S.A.) and forskolin (Calbiochem, San Diego, CA, U.S.A.) were prepared by dissolving these compounds in ethanol at 1 mg/ml (2·84 mM) for prostaglandins and 10 mM for forskolin. These drugs were diluted daily into fresh Locke solution. Ethanol, at concentrations as high as 10 μl/ml, did not measurably affect the slow a.h.p. Serotonin (5-HT, Sigma) and PGD_2 were pressure-applied from a focally situated micropipette, as described by Fowler *et al.* (1985*a*).

An Axoclamp-II amplifier (Axon Instruments, CA, U.S.A.) was used in the discontinuous current injection mode with a switching frequency between 3 and 6 kHz. The headstage voltage

was continuously monitored to ensure that it settled completely prior to sampling. The output of the sample-and-hold amplifier was low-pass filtered at approximately $\frac{1}{10}$ the sampling frequency. Thin-walled glass microelectrodes (0·9 mm i.d., 1·2 mm o.d.; Frederick Haer) were filled with a mixture of 1 M-potassium acetate and 2 M-KCl (pH 7·2, 20–50 MΩ). The slow a.h.p. appeared to be more stable when the electrodes contained acetate; the addition of KCl decreased the electrode resistance, thereby reducing the settling time of the headstage voltage during discontinuous mode operation.

Amplifier outputs were displayed on-line with an oscilloscope and chart recorder (Gould, 2400) and were stored on FM tape (Racal, 4DS). Analog signals were filtered at half the sampling frequency to eliminate aliased noise and digitized for analysis on a DEC PDP-11/23 computer system using a DT2781 A/D interface. Analysis and display of the digitized responses were performed on a digital storage oscilloscope (Hewlett–Packard, 1345A) and a digital X–Y plotter (Hewlett–Packard, 7470A). All Figures are from photographs of X–Y hard-copies. Data are summarized as average values ± the standard error of the mean (S.E.M.).

RESULTS

Selective inhibition of the slow a.h.p. by prostaglandins

Previously, we reported that PGE_1 and PGD_2 blocked the slow a.h.p. in rabbit nodose neurones (Fowler *et al.* 1985*a*, *b*). Presently, we report in greater detail the effects of focally applied PGD_2 (1, 10 and 100 nM). Prostaglandin D_2 produced three concentration-dependent effects: (1) inhibition of the slow a.h.p. (50% reduction in area estimated at 21 nM); (2) depolarization of the resting membrane potential (V_m; < 10 mV); and (3) an increase in resting membrane resistance (R_m) (maximum increase of 28%). These effects showed a gradual onset, reaching a steady-state level after 1–2 min exposure to PGD_2. The depolarization and increase in R_m were observed only in association with inhibition of the slow a.h.p., and were never seen in neurones without slow a.h.p.s. We suggest that the Ca^{2+}-dependent K^+ conductance ($g_{K(Ca)}$) responsible for the slow a.h.p. is also partially active at rest, and that inhibition of this tonically active $g_{K(Ca)}$ produces the depolarization and conductance decrease observed with PGD_2 application.

By calculating the increase in conductance (g_{ahp}) during the slow a.h.p., the effects of PGD_2 on the slow a.h.p. could be measured independently of changes in R_m. The g_{ahp} was calculated using the equation:

$$g_{ahp}(t) = \frac{g_m V_{ahp}(t)}{(E_{ahp} - V_m) - V_{ahp}(t)}, \quad (1)$$

where $g_{ahp}(t)$ is the conductance at time t, $V_{ahp}(t)$ is the amplitude of the slow a.h.p. at time t, V_m is the pre-spike membrane potential, g_m is the resting membrane conductance and E_{ahp} is the reversal potential of the slow a.h.p. (−80 mV, $n = 4$; cf. Fowler *et al.* 1985*a*). A similar method has been used to calculate the conductance underlying a fast, Ca^{2+}-dependent spike a.h.p. in cat spinal motoneurones (Baldissera & Gustafsson, 1974*a*; Barrett, Barrett & Crill, 1980). The membrane time constant (τ_m) was not included in the calculation since the time constants of onset (τ_{on}) and decay (τ_{off}) of the slow a.h.p. are greater than 10 times τ_m (Wonderlin, 1986).

Prostaglandin D_2 produced a concentration-dependent reduction in the peak, duration and area of g_{ahp} with an average maximum inhibition at 100 nM (expressed as percentage of the control response) of 17, 42 and 9%, respectively ($n = 12$). The PGD_2 concentration producing 50% inhibition (IC_{50}) was estimated by a linear least-

squares fit to the data, resulting in IC_{50} values for peak, duration and area of 21, 70 and 6 nM, respectively. The data summarized above were based on slow a.h.p.s elicited by a single action potential. The surmountability of the block by PGD_2 was examined by using 1–16 spikes to elicit the slow a.h.p. When inhibition of the single-spike slow a.h.p. was incomplete, there was partial surmountability of the block. However, when the a.h.p. produced by a single spike was completely blocked, no appreciable surmountability of the block was observed with increased spike number.

We also compared the inhibitory actions of PGE_2 and $PGF_{2\alpha}$ to PGD_2. Structurally, PGD_2, PGE_2 and $PGF_{2\alpha}$ have identical aliphatic side chains and differ only in the substitution of their polar, cyclopentane head groups. Bath application of 100 nM-PGE_2 or $PGF_{2\alpha}$ reduced the peak $g_{K(Ca)}$ to 2% ($n = 6$) and 80% ($n = 4$) of control, respectively. Thus, PGE_2 was similar to PGD_2 in its inhibition of the slow a.h.p., whereas $PGF_{2\alpha}$ was only slightly effective.

We considered the activation of adenylate cyclase and the elevation of intracellular cyclic AMP levels to be a likely subcellular mechanism responsible for prostaglandin-induced inhibition of the slow a.h.p. because: (1) PGD_2 (1–100 nM) has been shown to activate adenylate cyclase in neuroblastoma cells (Shimizu, Mizuno, Amano & Hayaishi, 1979) and (2) exposure to forskolin, an activator of adenylate cyclase (Seamon & Daly, 1981), inhibits a slow, long-lasting Ca^{2+}-dependent spike a.h.p. in hippocampal CA3 neurones (Madison & Nicoll, 1982) and a slow, Ca^{2+}-dependent a.h.p. in guinea-pig myenteric neurones (Nemeth, Zafirov & Wood, 1984). In rabbit nodose neurones, bath application of forskolin (25–500 nM) produced a concentration-dependent inhibition of the slow a.h.p. with an IC_{50} estimated to be 30 nM and complete block occurred at 1–10 μM. The inhibition produced by forskolin was accompanied by a slow depolarization and increase in R_m and it resembled the inhibition produced by PGD_2 with regard to changes in peak, duration, and area of g_{ahp}. Although these experiments do not directly demonstrate a role of adenylate cyclase activation in the block of the slow a.h.p. by prostaglandins, they do indicate that activation of adenylate cyclase is sufficient to produce block of the slow a.h.p. Additional evidence suggesting that the effect of PGD_2 might be mediated by activation of adenylate cyclase has been obtained from preliminary biochemical studies that have shown that both PGD_2 and forskolin produce a concentration-dependent elevation of cyclic AMP levels in isolated rabbit nodose ganglia (our unpublished observations).

Effects of inhibition of the slow $g_{K(Ca)}$ on the voltage–current relationship in nodose neurones

The *V–I* relationships of nodose neurones were studied by examining the voltage transients produced by injection of rectangular current steps (200 ms duration) using discontinuous current clamp. The *V–I* properties of a representative neurone before and after block of the slow a.h.p. by PGD_2 are shown in Fig. 1. This neurone exhibited some rectification, i.e. an increase in membrane conductance with depolarization relative to rest (Fig. 1*B* and *C*). This rectification was constant from the completion of the charging of the membrane capacitance to the end of the current pulse. Time-dependent rectification, such as the delayed-onset inward rectification

with hyperpolarizing steps, was never observed in C neurones. *V–I* profiles similar to that illustrated in Fig. 1 were observed in all C neurones (with or without slow a.h.p.s) in the present study ($n = 46$) and are typical of C-fibre neurones in rabbit nodose (Stansfeld & Wallis, 1985) and rat dorsal root ganglia (Harper & Lawson, 1985). When an action potential was elicited by a depolarizing step, slow a.h.p.

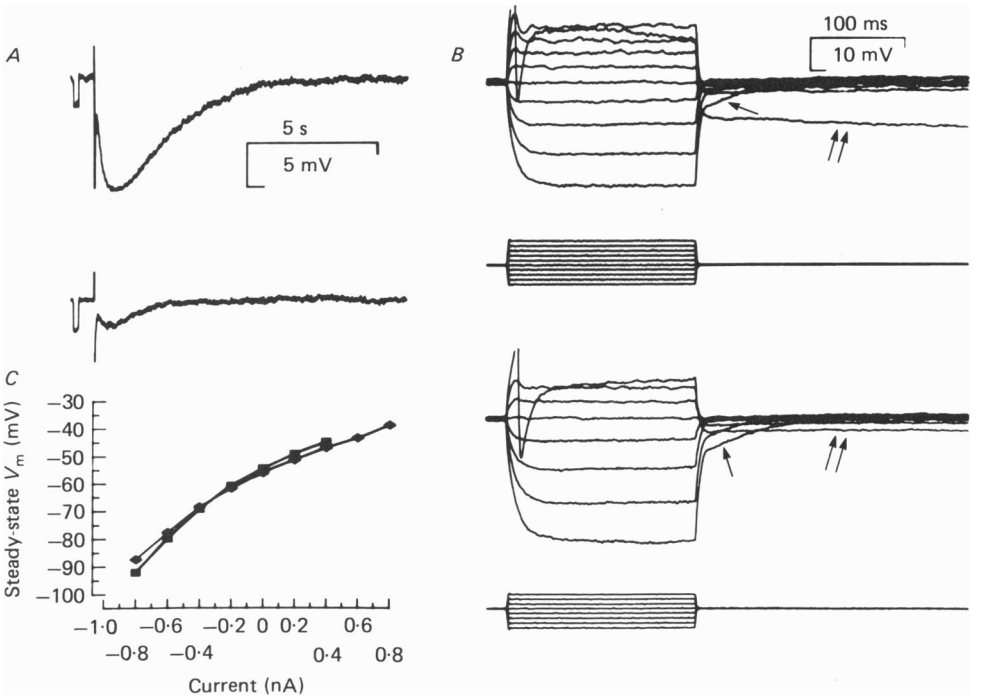

Fig. 1. Influence of the slow a.h.p. on *V–I* relationships in a single nodose neurone. *A*, single-spike slow a.h.p. before (upper trace) and after (lower trace) exposure to PGD_2 (100 nM). Resting membrane potential is −56 mV. The deflection preceding the spike is an electrotonic voltage transient produced by a 100 pA hyperpolarizing constant-current pulse. *B*, *V–I* relationships before (upper panel) and after (lower panel) inhibition of the slow a.h.p. In each panel, the upper set of traces are the voltage records and the lower set of traces are the current records (200 ms steps, 200 pA increments) An action potential triggered a slow a.h.p. which can be seen as a hyperpolarizing tail after the step (double arrow). This neurone also exhibited a hyperpolarized notch (single arrow) in its recovery from large hyperpolarizing current steps (see text). *C*, steady-state voltage responses measured at 150 ms after the beginning of the current steps are plotted as a function of current amplitude. The rectification observed prior to inhibition of the slow a.h.p. by PGD_2 (◆) is not changed following inhibition of the slow a.h.p. (■), although there is a small increase in membrane resistance.

neurones exhibited a slowly developing hyperpolarization with a delayed onset that minimally affected the voltage response during a 200 ms step (compare deflections before and after blockade of the slow a.h.p.), but was evident as a hyperpolarizing tail following the depolarizing step (double arrow in Fig. 1*B*). Some C neurones exhibited a small hyperpolarizing notch during their repolarization from strongly hyperpolarized potentials (single arrow in Fig. 1*B*). The ionic conductance responsible for this notch was not positively identified, but it could correspond to a

transient outward current (A-current) that has been identified in rat nodose neurones (Cooper & Shrier, 1985) and that we have observed in voltage-clamped rabbit nodose neurones (our unpublished observation).

Inhibition of the slow a.h.p. by prostaglandins or by forskolin was usually accompanied by both an increased membrane resistance and a membrane depolarization (Fig. 1, the depolarization is not shown in the Figure because the V_m was current clamped back to the pre-exposure resting V_m). No other effects of PGD_2 or forskolin on the *V–I* characteristics of slow a.h.p. neurones were observed. The *V–I* characteristics of C neurones without slow a.h.p.s were unaffected by prostaglandins or forskolin.

Influence of slow a.h.p. inhibition on spike train interval

We predicted that the maximum spike firing rate of slow a.h.p. neurones might be regulated by an increased interspike refractory period resulting from the slow a.h.p. Because the somata of nodose neurones do not exhibit spontaneous spike firing *in vitro*, we simulated different spike firing rates by injecting trains of brief current pulses (2 ms) at interstimulus intervals (i.s.i.) between 100 ms and 10 s. Brief current pulses were used to avoid prolongation of the normal, spike-activated, voltage-gated Ca^{2+} influx; the action potential duration of C-fibre neurones is 2·5 ms (Jaffe & Sampson, 1976; Stansfeld & Wallis, 1985). For these experiments the strength of the depolarizing current pulses was set at 20% above that required to elicit a single spike, thereby providing a small safety factor against random spike failure. The ability of neurones to respond to a stimulus train was quantified as the percentage of current pulses that successfully elicited action potentials:

$$\text{percentage success} = \frac{N-1}{(\text{no. pulses for } N \text{ spikes})-1} \times 100, \quad (2)$$

where N is the number of action potentials evoked by the stimulus train. Typically, the number of current pulses required to elicit five spikes during the steady-state response at each frequency was measured.

In the frequency range 0·1–10 Hz, slow a.h.p. neurones exhibited a decreased percentage success to stimuli at shorter i.s.i.s whereas the percentage success in neurones without slow a.h.p.s was constant at 100% for all i.s.i.s (Fig. 2*C*). The frequency dependence of spike-firing success *versus* i.s.i. in slow a.h.p. neurones was linear when plotted on log–log co-ordinates. Therefore, a linear, least-squares fit to these data could be used to estimate the interspike refractory period. If the interspike interval is an integral factor of the refractory period, then the refractory period can be calculated using:

$$\text{refractory period} = \frac{\text{i.s.i.} \times 100}{\text{percentage success}}. \quad (3)$$

However, if the interspike interval is not an integral factor of the refractory period, the refractory period will be overestimated with an error value less than the i.s.i. The magnitude of the error decreases with shorter i.s.i. values; therefore, a line was fitted by least squares to the data points at the smaller (< 1000 ms) i.s.i.s tested. Because the refractory period is equal to the i.s.i. when the percentage success = 100, the duration of the refractory period could be estimated as the i.s.i. value at which the

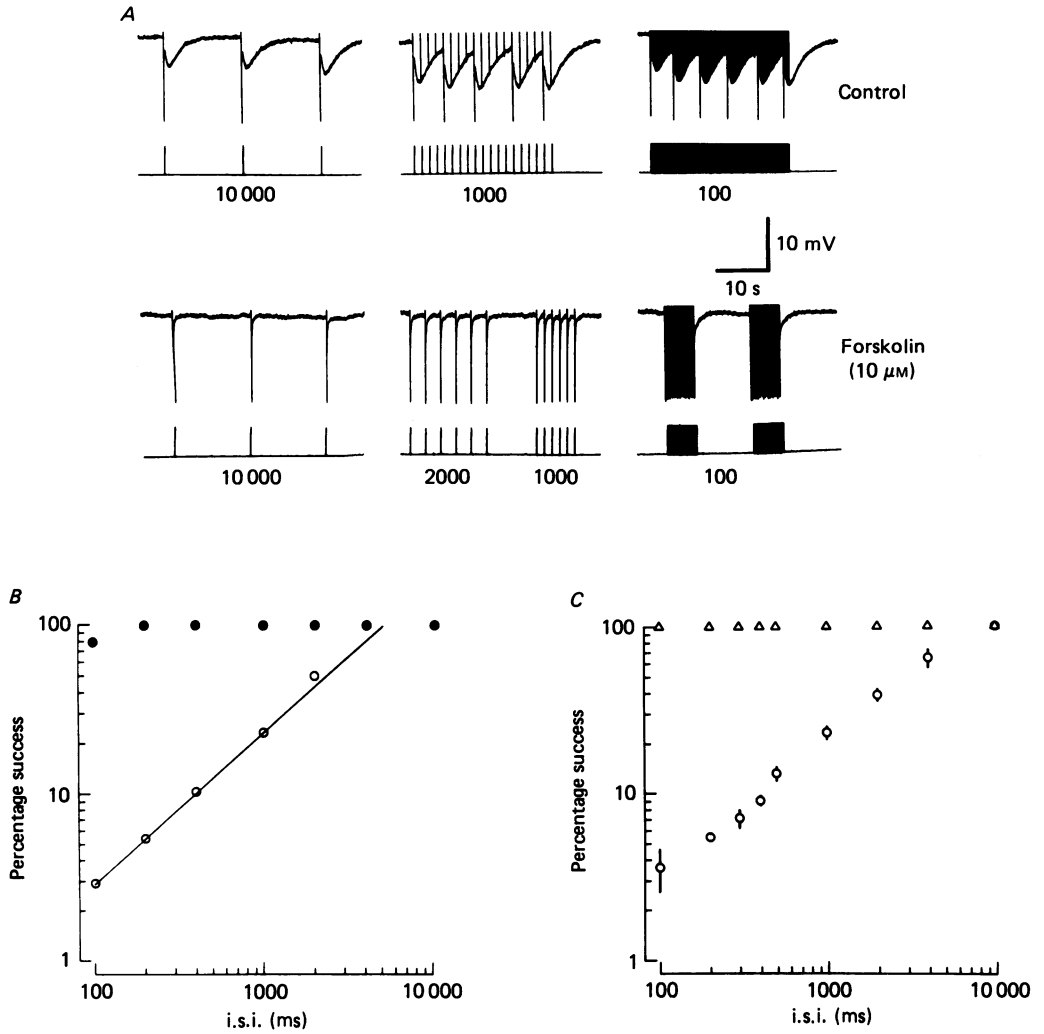

Fig. 2. Inhibition of the slow a.h.p. by 10 μM-forskolin increases the ability to respond to stimulus trains. *A*, records showing stimulus trains before (upper) and after (lower) exposure to 10 μM-forskolin, respectively. In each pair of records, the upper trace is the voltage record and the lower trace is the current record. Records for i.s.i.s of 10000, 1000 and 100 ms are shown on the same time-scale. Spikes are indicated by the downward deflections produced by fast a.h.p.s; positive-going spike components are truncated. Inhibition of the slow a.h.p. decreased the spike failures at 10000, 2000, 1000 and 100 ms i.s.i.s. *B*, plot of percentage success *versus* i.s.i. on log–log co-ordinates for records shown in *A*. The control responses (○) exhibited a frequency-dependent decrease in spike firing success with an estimated refractory period (defined in the text) in this neurone of 4900 ms. Inhibition of the slow a.h.p. (●) eliminated the frequency dependence. *C*, plot of percentage success (mean ± S.E.M) *versus* i.s.i. for slow a.h.p. neurones (○, $n = 8$) and neurones not exhibiting slow a.h.p.s (△, $n = 3$). Neurones without slow a.h.p.s exhibited no stimulus failures at i.s.i.s between 100 and 10000 ms, while slow a.h.p. neurones demonstrated a decreased ability to respond to stimulus trains at i.s.i. less than 10000 ms. Where the S.E.M. bars are absent, the S.E.M. is smaller than the symbol.

predicted Y value equalled 100% success (Fig. 2*B* and *C*). Following this method, the data were generally well fitted; the average correlation and slope between percentage success and i.s.i. was $0{\cdot}992 \pm 0{\cdot}003$ and $0{\cdot}893 \pm 0{\cdot}034$, respectively. Thus, for a stimulus strength of 120% of the single-spike threshold, the average estimated refractory period in slow a.h.p. neurones was 6298 ± 1108 ms ($n = 8$), corresponding to a maximum spike firing rate of 0·16 Hz. This method probably overestimates the refractory period because the slope of the best-fit lines was, on average, less than 1.

Following block of the slow a.h.p. by PGD_2 or by forskolin (Fig. 2*A*), spike trains could be elicited by stimulation at frequencies up to 10 Hz. The increase in firing ability was proportional to the degree of block of the slow a.h.p. In cases of partial block by prostaglandins, the enhancement of spike firing ability lessened with increasing stimulus frequency, perhaps as a result of the surmountability of a partial block. However, when the slow a.h.p. was completely blocked, as was typical with forskolin, the enhancement of firing ability was not diminished at higher frequencies. Bath application of prostaglandins or forskolin to C neurones without a slow a.h.p. did not alter their ability to respond to stimulus trains.

Response of slow a.h.p. neurones to paired depolarizing current ramps

We examined the influence of the slow a.h.p. on responses to phasic application of longer-duration stimuli, such as pairs of brief, depolarizing current ramps separated by inter-ramp intervals (i.r.i.) of 500–10000 ms. Thus, the first ramp was used to activate a slow a.h.p. and the second ramp was presented during the time course of the slow a.h.p. For each ramp we measured both the current threshold for activation of the first spike and the number of spikes activated during the ramp. Comparison of these measures before and after inhibition of the slow a.h.p. was used to determine how the slow a.h.p. influenced pre- and post-spike excitability.

Linear current ramps (slope = 1 nA/10 ms) were injected using the discontinuous current-clamp mode. The ramp duration was adjusted to evoke two to five action potentials with the first ramp, usually requiring 4–7 nA current at the peak of the ramp. In four neurones without slow a.h.p.s, ramps separated by a 2000 ms i.r.i. produced the same number of action potentials. The average current threshold for the first spike of the first ramp (T_1) was 750 ± 17 pA. The average current threshold for activation of the first spike of the second ramp (T_2) was $102{\cdot}0 \pm 0{\cdot}9$% of T_1. In most cases the paired responses were superimposable. A similar absence of interaction between the first and second ramps was observed for i.r.i.s between 100 and 5000 ms, indicating that activation of a train of spikes by the first ramp does not alter excitability during this time domain.

Slow a.h.p. neurones exhibit a very different response to paired ramps (Fig. 3). In eight slow a.h.p. neurones, the average T_1 was 1251 ± 195 pA at a 2000–3000 ms i.r.i., and T_2 was, on average, 338 ± 35% of T_1. Also, the average number of spikes elicited by the second ramp was reduced to $19{\cdot}2 \pm 6$% of the number of spikes activated by the first ramp. In some neurones, action potentials could not be elicited by a second ramp presented at an i.r.i. corresponding to the peak of the slow a.h.p., even at greater current intensities and more depolarized potentials than those required to produce multiple action potentials with the first ramp. The decreased excitability during the slow a.h.p. results from the hyperpolarization and increased membrane

conductance, the latter of which decreases the rate of depolarization and increases the inhibitory influence of accommodation to the depolarizing stimulus. These experiments demonstrate that activation of the slow a.h.p. by the first ramp exerts a powerful depression of excitation by a second stimulus presented during the time domain of the slow a.h.p.

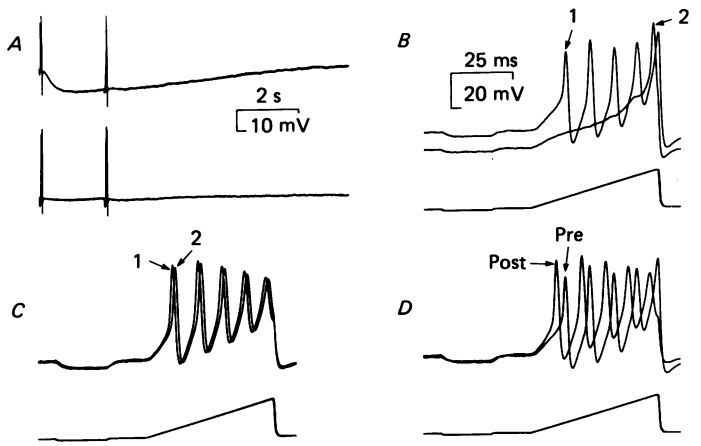

Fig. 3. *A*, voltage responses to paired depolarizing current ramps (4 nA/40 ms, 2100 ms i.r.i.) before (upper trace) and after (lower trace) inhibition of the slow a.h.p. by 100 nM-PGD_2. Resting V_m prior to the first ramp is −59 mV. *B*, overlay of the control first and second ramped responses from *A* prior to exposure to PGD_2 at a faster sweep speed. The calibration is the same in *B*, *C* and *D*. The first spike in each ramp is identified by a numbered arrow. Each ramp is preceded by a 100 pA current step which provides a measure of R_m and membrane time constant at the beginning of each ramp. With activation of a slow a.h.p. by the first ramp, the second ramp activates only a single spike, which is delayed relative to the first spike of the first ramp. *C*, overlay of the first and second ramped responses in *A* following block of the slow a.h.p. by PGD_2. The first and second ramps evoked the same number of action potentials with similar current thresholds for each first spike. *D*, comparison of the first ramp responses before (Pre) and after (Post) block of the slow a.h.p. Inhibition of the slow a.h.p. did not change the number of spikes activated by the first ramp, but did increase the spike amplitude and decrease the current threshold for spike activation. These changes probably result from the increase in the resting membrane resistance.

Inhibition of the slow a.h.p. by prostaglandins or by forskolin produced a large increase in excitability (Fig. 3*C*), with both ramps eliciting the same number of action potentials. The average value of T_2 was reduced to only 109 ± 4% of T_1. The increased membrane resistance produced a 30% decrease in T_1, perhaps as a result of the greater rate of depolarization and the concomitant reduction in accommodation to the stimulus. Inhibition of the slow a.h.p. did not change the number of spikes activated by the first ramp (Fig. 3*D*). The responses of C neurones without slow a.h.p.s to paired depolarizing ramps were not affected by either prostaglandins or forskolin.

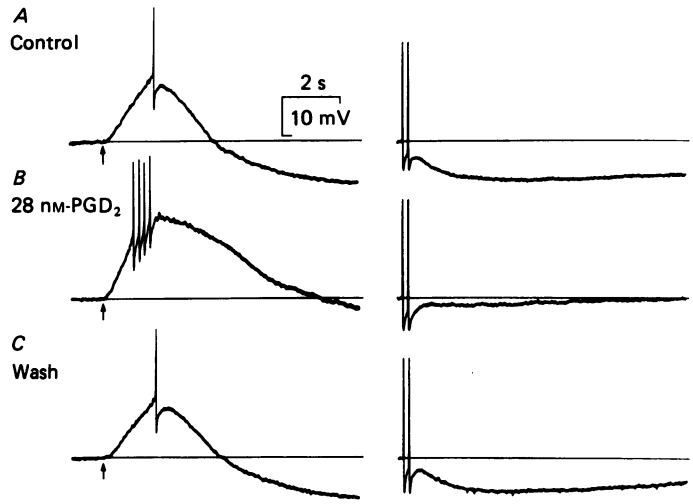

Fig. 4. Prostaglandin D_2 potentiates the excitatory effects of serotonin (5-HT). *A*, the left trace shows the control depolarization produced by focally applied 5-HT. The vertical arrow under the trace indicates the initiation of a 100 ms pressure application of 30 μM-5-HT. The 5-HT produced a rapid depolarization, an action potential and a slow hyperpolarization. The corresponding two-spike slow a.h.p. activated by current injection in the same neurone is shown in the right trace. The pre-stimulus membrane potential is -50 mV. *B*, bath application of 28 nM-PGD_2 potentiated the excitatory response to the same amount of 5-HT (left trace) concurrently with inhibition of the slow a.h.p. activated by current injection (right trace). Two factors responsible for increasing the number of spikes elicited by the depolarizing response to 5-HT include the weaker accommodation resulting from an increase in membrane resistance due to blockade of a tonically active $g_{K(Ca)}$ and the absence of the post-spike hyperpolarization. *C*, both the depolarizing response to 5-HT (left trace) and the slow a.h.p. activated by current injection (right trace) returned to control levels with wash in normal Locke solution. Similar results were observed in four additional experiments.

DISCUSSION

The experiments reported above demonstrate that the $g_{K(Ca)}$ activated during the slow a.h.p. produces a post-spike refractory period which, when eliminated by inhibition of the slow a.h.p., dramatically increases excitability. In addition to PGD_2 and PGE_2, the slow a.h.p. is inhibited by bradykinin (Weinreich, 1986), histamine and arachidonic acid (Wonderlin, 1986). These substances are important autocoids, acting, for example, as mediators of allergic hypersensitivity responses when immunologically released from mast cells (Schleimer, MacGlashan, Schulman, Peters, Adams, Adkinson & Lichtenstein, 1982). Hyperexcitability of visceral sensory neurones following inhibition of the slow a.h.p. might contribute to the pathophysiological responses produced by these autocoids. Identical excitatory effects were observed whether PGD_2 or a general activator of adenylate cyclase, forskolin, were used to block the slow a.h.p. This suggests that the ability to activate adenylate cyclase might be an important property of substances that enhance visceral sensory excitability.

Our observations indicate that marked differences in excitability between subpopulations of C neurones can be attributed to the presence of the slow a.h.p.

Based on the measurements presented above, all C neurones exhibited similar excitability following complete inhibition of the slow a.h.p.

Unfortunately, the mechanism of excitation produced by prostaglandins in nodose neurones does not shed light on the excitatory effects of prostaglandins in other neuronal populations. In *Helix* neurones, prostaglandins increase the spontaneous spike firing rate of A-neurones, presumably as a result of inhibiting a fast, Ca^{2+}-dependent spike a.h.p. (Madden & Van der Kloot, 1984). However, the fast, Ca^{2+}-dependent spike a.h.p. in *Helix* neurones is probably homologous with the fast, Ca^{2+}-dependent spike a.h.p. in nodose neurones which is not affected by prostaglandins (Fowler *et al.* 1985*a*). Prostaglandins also increase the excitability of dorsal root ganglion neurones in culture (Baccaglini & Hogan, 1983) and depolarize neurones from the rabbit superior cervical ganglion (Dun, 1980) and the rat cerebellum (Kimura, Okamoto & Sakai, 1985). However, the Ca^{2+}-dependent K^+ currents present in these neurones are probably not homologous with the slow a.h.p., and we observed no effects of prostaglandins on membrane excitability in nodose neurones that did not occur concurrently with inhibition of the slow a.h.p.

Does somal excitation provide a model for excitation of sensory terminals by prostaglandins?

The control of the excitability of the somata of nodose C-fibre neurones by the Ca^{2+}-dependent slow a.h.p. provides a preliminary model for the regulation of excitability at sensory terminals. There is a precedent for similar physiological properties being shared by the somata and sensory terminals of some primary visceral afferent neurones in the petrosal ganglion (Belmonte & Gallego, 1983). If the slow a.h.p. is also present in sensory terminals, it could profoundly influence sensory transduction by controlling the frequency-dependent encoding of graded stimuli into action potentials which are conducted to the C.N.S. Our *in vitro* observations of potentiation of depolarizing stimuli by prostaglandins might provide an explanation for the prostaglandin-mediated sensitization of lung afferents that has been observed *in vivo*. Prostaglandins of the E series, either injected into the right atrium or aerosolized into the lungs of anaesthetized dogs, increase the spontaneous firing rate of isolated vagal pulmonary and bronchial C-neurone fibres (Coleridge, Coleridge, Ginzel, Baker, Banzett & Morrison, 1976; Coleridge, Coleridge, Baker, Ginzel & Morrison, 1978). According to these authors, the excitation is independent of interactions with airway smooth muscle and results from direct activation or sensitization of C-fibre receptors. The range of increased spontaneous spiking activity they observed, approximately 5–10-fold (i.e. from 0·6–1 to 5–10 Hz), is in the range of the increased excitability reported in the present study.

The slow a.h.p. might also regulate the sensitivity of sensory neurones to chemical stimulation. In rabbit lung, some C-neurone sensory endings are distributed in close association with neuroepithelial complexes that contain serotonin (Lauweryns, Van Ranst & Verhofstad, 1986), a potent stimulant of the somata of rabbit nodose C neurones (Higashi & Nishi, 1982). The data illustrated in Fig. 4 demonstrate that inhibition of the slow a.h.p. can markedly sensitize visceral C neurones to stimulation by serotonin. Thus, the presence of the slow a.h.p. could play a prominent role in regulation of lung sensory function.

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