Effects of pinaverium on voltage-activated calcium channel currents of single smooth muscle cells isolated from the longitudinal muscle of the rabbit jejunum

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1 Smooth muscle cells of the longitudinal muscle of the rabbit jejunum were dispersed by enzyme treatment and recordings of membrane current were made in the whole-cell mode by patch clamp technique. The action of pinaverium bromide on the voltage-dependent inward current of single isolated smooth muscle cells was studied in solutions containing normal concentrations of calcium or high concentrations of barium at room temperature.

2 Pinaverium reduced the voltage-dependent inward current with an IC_{50} of $1.5 \mu M$. This IC_{50} is similar to those of verapamil, diltiazem and flunarizine on these cells as described by others. Occasionally evidence of a potentiating action of pinaverium on the inward current was seen.

3 Repetitive stimulation of the cells did not increase blockade of inward current by pinaverium unlike the use-dependent blockade seen with verapamil, methoxyverapamil, and diltiazem in these and in other smooth muscle cells.

4 The inactivation of inward current was studied by holding at various potentials for 2 or 10 s before evoking inward current. The voltage at which current was 50% available was changed very little by pinaverium although other calcium entry blockers, for example the dihydropyridines, have been reported to produce appreciable negative shifts which indicate considerable voltage-dependence of their blockade. This may indicate that pinaverium has similar affinities for the closed available and inactivated calcium channel states so that blockade is not appreciably voltage-dependent.

Introduction

Pinaverium bromide has been used to treat hypermotility disorders of the intestine including irritable bowel syndrome (see Breteau & Foussard-Blanpin, 1980 for references). Pinaverium reduced the contractile activity of canine gastrointestinal muscle and slowed the propagation of the myoelectric complex (Itoh & Takahashi, 1981; Grenier *et al.*, 1983). In experiments on isolated smooth muscle preparations, pinaverium inhibited contractions of intestinal muscle to nerve stimulation, barium chloride and acetylcholine. The inhibition was non-competitive and was antagonized by increasing the calcium concentration of the bathing solution (Breteau & Foussard-Blanpin, 1980; Baumgartner *et al.*, 1985). However, pinaverium has little blocking activity at muscarinic receptors (Breteau *et al.*, 1975) suggesting that its spasmolytic action is exerted in some other way. Pinaverium binds to calmodulin (Ronca-Testoni *et al.*, 1985) but this does not seem to be the basis of its action (Wuytack *et al.*, 1985). Rather it seems to have calcium antagonist (calcium entry blocking) properties although at higher concentrations (10^{-5} M) it may also inhibit intracellular calcium store release. Pinaverium reduced the frequency, amplitude, maximum rate of rise and fall of action potentials in guinea-pig ileum and in uterus without much effect on the resting potential (Droogmans *et al.*, 1983; Mironneau *et al.*, 1984; Wuytack *et al.*, 1985). Contractions of smooth muscle of rabbit ear artery to noradrenaline are much less sensitive to the blocking action of pinaverium than are contractions to high potassium solutions. Resting calcium uptake is not affected by pinaverium, but the calcium uptake in response to high potassium solution and the inward current in myometrial strips are markedly depressed (Droogmans *et al.*, 1983; Mironneau *et al.*, 1984).

The results strongly suggest that pinaverium has calcium antagonist, or calcium-entry blocking, properties. The experiments described in this paper were designed to examine these properties in more detail in single, dispersed, voltage-clamped intestinal smooth muscle cells. A number of calcium antagonists have been studied for their effects on the inward current in these cells: these include dihydropyridines (Droogmans & Callewaert, 1986; Bolton *et al.*, 1987; Terada *et al.*, 1987a,b), flunarizine (Terada *et al.*, 1987c) and methoxyverapamil (D600) or verapamil (Ohya *et al.*, 1987; Terada *et al.*, 1987a). The actions of pinaverium were compared with the known actions of other calcium antagonists on the voltage-dependent inward current in these cells.

Methods

Cells were dispersed from the longitudinal smooth muscle of the rabbit jejunum. Four pieces ($\approx 3 \times 4$ mm) were cut and incubated in a low Ca^{2+} ($2 \mu M$ added) physiological salt solution (PSS) for 10 min at $37^\circ C$. They were subsequently incubated in nominally Ca^{2+} -free PSS containing papain (5 mg ml^{-1}), dithiothreitol (5 mM) and bovine serum albumin (BSA, 5 mg ml^{-1}) for 10–15 min and then collagenase (0.2 mg ml^{-1} , Sigma type XI), trypsin inhibitor (1 mg ml^{-1}) and BSA (5 mg ml^{-1}) for 5–10 min, both at $37^\circ C$. After dilution of the enzymes, cells were agitated in the nominally Ca^{2+} -free PSS. The selected suspension of cells was then centrifuged at $100g$ for 2 min and the pellet re-suspended in $\approx 0.8 \text{ mM}$ Ca^{2+} containing PSS. Cells were maintained at $4^\circ C$ and used within 12 h. All experiments were at room temperature (20 – $25^\circ C$).

The PSS in the bath had the following ionic composition (mM): Na^+ 125, K^+ 6, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 138.4, glucose 10.5, HEPES 10, and was titrated to pH 7.2 with NaOH. The high-barium bath solution had the composition (mM): Ba^{2+} 110, Cl^- 220, HEPES 10, and was titrated to pH 7.2 with NaOH. The pipette (= intracellular) solution had the composition (mM): Na^+ or Cs^+ 130, Mg^{2+} 3.2, TEA⁺ 10, Cl^-

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142.4, ATP 2, glucose 11.5, HEPES 5, EGTA 2, and was titrated to pH 7.2 with tetraethylammonium hydroxide.

Cells were voltage-clamped using the whole-cell configuration of the patch-clamp technique (Hamill *et al.*, 1981). The current-voltage converter was a List EPC-7 and analogue signals were recorded on FM-tape (Racal) at a tape-speed of 7.5 in s^{-1} . Patch pipettes ($2\text{--}4 \text{ M}\Omega$) were made from borosilicate glass. Signals were digitized at 2 kHz using a CED1401 ADC interface in conjunction with a BBC microcomputer and displayed on a graph plotter (Hewlett Packard), or were displayed directly on chart paper (Gould 2400). In both instances, signals were first low-pass filtered at 1 kHz (8-pole Bessel, Barr & Stroud). Functions were fitted by minimizing the unweighted sum of the squares of the deviations using a Marquardt-Levenberg algorithm. Leak current was estimated by applying five different short (0.1 s) and small ($<20 \text{ mV}$) hyperpolarizing steps from the holding potential. It was subtracted either by an analogue circuit, which subtracted a scaled linear component directly, or by fitting the mean leakage currents elicited by the hyperpolarizing steps with a straight line and subtracting this function from the currents elicited by depolarizing voltage-steps. Leak subtracted currents are shown in the figures.

Pinaverium bromide (Dicetal) is 4-(6-bromoveratryl)-4-[2-(2-(10-norpinan-2-yl)ethoxy)ethyl] morpholinium bromide and was obtained from Latema (LTM) Paris. It was diluted for use from a 10 mM stock solution in 100% ethanol. Tetraethylammonium (TEA) hydroxide was obtained from Sigma.

Results

In cells held at negative potentials (-60 to -90 mV) under voltage clamp, stepping to potentials positive to -60 mV elicited inward calcium current which was relatively well sustained for smaller depolarizations but which inactivated very substantially for larger depolarizations lasting 0.5 s (Bolton *et al.*, 1987). This current was reduced but not completely abolished by pinaverium ($1\text{--}100 \mu\text{M}$) (Figure 1). The IC_{50} for pinaverium was about $1.5 \mu\text{M}$ (Figure 2). Peak current was reduced at all potentials (Figure 2a) but the time course of inactivation was not greatly affected (Figure 1).

In cells held under voltage clamp for a period, inward current evoked at various intervals decreased in size although in the best cells this decrease was small. However, in some cells the application of pinaverium ($0.01\text{--}0.1 \mu\text{M}$) occasionally

Figure 1 Effect of pinaverium on inward calcium current in a cell bathed in physiological salt solution (PSS) containing 2.5 mM calcium. Leak current was subtracted in all cases (see Methods) and for all subsequent figures. The cell was held at -70 mV in the absence (control) and then in the presence of $1 \mu\text{M}$ pinaverium and stepped to the indicated test potentials.

Figure 2 (a) Current-voltage relationship for peak inward calcium current activated from a holding potential (HP) of -70 mV . Control ($\square$), block after 5 min in the presence of $3 \mu\text{M}$ pinaverium ($\blacksquare$) and partial recovery 5 min after wash-out of drug ($\triangle$) are shown. Ordinate: peak inward current. Abscissa scale: membrane potential. (b) Inhibition by pinaverium of peak inward current evoked in a series of single cells in solution containing 2.5 mM calcium concentration. The dotted line indicates the IC_{50} concentration ($1.5 \mu\text{M}$) and the numbers of cells involved are indicated by each point (mean with s.e. mean shown by vertical lines). Cells were held at -70 mV and stepped to $+10 \text{ mV}$.

increased the current either close to threshold or by producing a small negative shift in the current-voltage relationship (Figure 3). Although in Figure 3 a -90 mV holding potential was used, potentiation was also seen in another cell held at -70 mV . Since the effect was quite marked on some occasions whereas a slow decline in current size was normally seen in the absence of pinaverium, it seemed possible that the potentiating effect resembles that observed with nifedipine (Aaronson *et al.*, 1988).

The block by pinaverium was not conspicuously use-dependent. If pulses (0.1 s) were applied every 20 s , the reduction of inward current was only slightly greater (57%)

Figure 3 Potentiation of inward current by pinaverium seen in the negative potential range in some cells. Current-voltage relationship for peak inward calcium current activated from a holding potential (HP) of -90 mV. Control ($\square$), increase in the presence of $0.01 \mu\text{M}$ ($\bullet$) and block in the presence of $10 \mu\text{M}$ ($\blacksquare$) pinaverium. Ordinate scale: peak inward current. Abscissa scale: membrane potential.

after 15 pulses than it was for the first pulse of a train (51%) (Figure 4). A similar result was obtained when normal solution (2.5 mM) calcium was substituted by a high-barium solution which causes the rate of inactivation of the current to slow (Bolton *et al.*, 1988). Reduction of peak current by $1 \mu\text{M}$ pinaverium was 16% after 16 pulses at 1 s intervals compared to 14% with the first pulse after a 2 min rest and 69% and 68% at the corresponding pulses in the presence of $10 \mu\text{M}$ pinaverium (Figure 5a). The smaller effect of $1 \mu\text{M}$ pinaverium in high-barium solution (Figure 5) compared to normal Ca solution (Figure 4) was not further investigated and may simply reflect experimental variation. The decline of current during a 2 s pulse in a solution containing 110 mM barium was also

Figure 4 Block of calcium current by pinaverium is not use-dependent. The cell was stepped to -10 mV for 0.1 s every 20 s from a holding potential of -70 mV when bathed in PSS containing 2.5 mM calcium concentration. Peak inward current during a train of pulses is plotted against time for the control ($\square$) and after 2 min at the holding potential in $1 \mu\text{M}$ pinaverium ($\blacksquare$).

Figure 5 Action of pinaverium on inward current in a high-Ba solution. In (a) the cell was stepped to $+20$ mV for 0.1 s every 1 s from a holding potential of -60 mV when bathed in 110 mM barium-solution. Peak inward current is plotted against time for the control ($\square$), and after 2 min at the holding potential in $1 \mu\text{M}$ ($\blacksquare$) and then in $10 \mu\text{M}$ pinaverium ($\blacktriangle$). (b) In the same cell, the effect of pinaverium on the rate of decay of inward current over 2 s at $+20$ mV is shown. The currents are each the average of 3 records. Bi-exponential functions with a constant were fitted (shown) and these had the time constants (τ) 0.22 and 9.1 s for the control, and 0.18 and 13.7 s in $1 \mu\text{M}$ pinaverium.

slightly affected; two exponentials could be fitted to the time course of the current in the control; the first was slightly accelerated and the second of these was moderately slowed in pinaverium (Figure 5b).

The availability of the inward current in 2.5 mM calcium was tested by measuring the absolute size of peak current evoked by a test potential of $+20$ mV after holding for 2 s at various conditioning potentials. The holding potential was -90 mV. More positive conditioning potentials than -90 mV reduced the availability of inward current, especially positive to -60 mV. The current was half available at $V_h = -39$ mV, and the slope, V_s , was 7.5 mV, when a Boltzmann equation of the form $I/I_{\max} = [1 + \exp\{(V - V_h)/V_s\}]^{-1}$ was fitted to the inactivating component of the current. V is the conditioning membrane potential and $I/I_{\max}$ the fraction of current available relative to that available from -90 mV. Pinaverium ($1 \mu\text{M}$) reduced the peak current evoked at $+20$ mV from all potentials with little change in V_h (-40 mV) or V_s (8.5 mV) (Figure 6) i.e. the fractional reduction in current was similar at all conditioning potentials.

Similar results were obtained in solutions where 2.5 mM Ca was replaced by 110 mM barium. Under these conditions the availability was shifted positively ($V_h = -7$ and -13 mV for 2 s and 10 s conditioning pulses) by about 30 mV (see also

Figure 6 The effect of pinaverium on the inactivation curves for the inward current (2.5 mM calcium in bathing solution). Inward current was elicited by stepping to +20 mV for 0.5 s. Cells were held at the conditioning voltage for 2 s, and 12.5 s was allowed for recovery from inactivation at the holding potential (HP) (-90 mV). Points represent peak inward current and they were fitted by the Boltzmann equation (see text). Inward current was 50% inactivated at -39 mV (control, $\square$) and at -40 mV in 1 μ M pinaverium ($\blacksquare$).

Aaronson *et al.*, 1988). Pinaverium (1 μ M) had a small effect on V_h (-19 mV) if a 10 s conditioning pulse was used (Figure 7b) although only a small change in V_h was seen (V_h = -9.6 mV) if a 2 s conditioning pulse was used (Figure 7a). Slope factors (V_s) were 16.7 and 10 mV (2 s and 10 s conditioning pulses) in controls and 10.9 and 12 mV in pinaverium.

Discussion

The results described in this paper indicate that pinaverium blocks the calcium currents in single isolated intestinal smooth muscle cells with a potency very similar to that of verapamil (Terada *et al.*, 1987a), diltiazem (Terada *et al.*, 1987a) and flunarizine (Terada *et al.*, 1987c) although it is much less potent than dihydropyridines such as nifedipine (Terada *et al.*, 1987a). Dihydropyridines such as KW-3049 (Terada *et al.*, 1987b) and CV-4093 (Okabe *et al.*, 1987) are also more potent in single vascular smooth muscle cells. When negative holding potentials (e.g. -80 mV) and/or low concentrations are used, dihydropyridine calcium entry blockers increase rather than decrease inward calcium or barium current (Okabe *et al.*, 1987; Terada *et al.*, 1987a; Aaronson *et al.*, 1988). Pinaverium also sometimes potentiated the inward current, although it is not a dihydropyridine and this result may indicate that it acts at the same binding site on the calcium channels as dihydropyridines or that potentiation is a more general feature of the mechanism of action of calcium entry blockers on the calcium channel. Potentiation could be masked in the case of certain drugs normally considered to be antagonists, by their much stronger blocking action on the channel.

Very little negative shift in the availability curve was observed with pinaverium even when 10 s conditioning pulses were used. Under these conditions dihydropyridines cause a distinct negative shift (Terada *et al.*, 1987a,b; Okabe *et al.*, 1987) as do diltiazem and verapamil (Terada *et al.*, 1987a). The lack of shift with pinaverium suggests that the drug has similar affinities for the closed available state and the inactivated state of the calcium channel; thus holding at a depolarized potential, which would substantially increase the

Figure 7 The effect of pinaverium on the inactivation of inward current (110 mM barium in bathing solution). Inward current was elicited by stepping to +30 mV for 0.5 s. Conditioning potential step-length was 2 s (a) and 10 s (b), and 7.5 s (a) and 4.5 s (b) were allowed for recovery from inactivation at the holding potential (-80 mV). Points are for peak inward current and the fitted Boltzmann equations indicated that 50% inactivation occurred at -7 mV (a) and -13 mV (b) for the controls (a, $\square$; b, $\square$), and -9.6 mV (a) and -19 mV (b) when in 1 μ M pinaverium (a, $\blacksquare$; b, $\bullet$).

fraction of channels in the inactivated state with a corresponding reduction in the fraction in the resting (closed) state, apparently did not increase affinity for the channel. In the case of other calcium entry blockers, this increasing affinity is seen as an increasing reduction of current as the holding potential is made less negative, a phenomenon which has led to the postulation of the 'modulated receptor hypothesis' (Bean, 1984; Bean *et al.*, 1986) to explain the phenomenon. Essentially this hypothesis invokes a much higher affinity of the drug for the inactivated as compared to the resting (closed) state of the calcium channel. However, in the case of D600, verapamil and possibly diltiazem, the negative shift in the availability curve may indicate entry into and block of the open channel as an alternative mechanism of blockade (Hering *et al.*, 1989).

D600, verapamil and diltiazem (Ganitkevich *et al.*, 1986; Terada *et al.*, 1987c; Hering *et al.*, 1989) differed from pinaverium in that blockade was increased by repetitive stimulation. D600 blockade is dramatically reversed even at 10^{-4} M by a 3 min rest period (Hering *et al.*, 1989) but no such rest recovery was seen with pinaverium. Nifedipine also showed no

use-dependent blockade if first equilibrated (Hering *et al.*, 1988) and blockade by nicardipine is increased little if at all by stimulation (Terada *et al.*, 1987a). This also seems to be true of other dihydropyridines in smooth muscle (Okabe *et al.*, 1987; Terada *et al.*, 1987b).

The results suggest that pinaverium blockade of calcium channels in single isolated cells of small intestine occurs by a mechanism which differs from those of other calcium entry blockers so far studied in detail on these cells. Pinaverium blockade was neither appreciably use-dependent nor voltage-dependent, which implies that it probably does not enter the channel in its open state to produce block as D600 or verapamil may do, and may combine with closed and inactivated channel states with similar affinities.

Two or more types of voltage-dependent calcium channel have been distinguished on the basis of their different unitary conductances and pattern of channel openings (e.g. Worley *et al.*, 1986; Benham *et al.*, 1987; Yatani *et al.*, 1987). In some smooth muscles it has been inferred that these separate populations contribute components to the voltage-dependent

whole-cell calcium current which can be identified by various voltage protocols. In the case of longitudinal muscle of rabbit small intestine, a more rapidly inactivating component of inward current becomes available if more negative holding potentials are used. However, it is uncertain whether this component represents current through a separate population of calcium channels (Bolton *et al.*, 1988). In the present experiments, no evidence for more than one component of voltage-dependent inward current was found on the basis of the sensitivity to pinaverium.

These results indicate that pinaverium is a calcium entry blocking drug which is active in blocking the voltage-dependent calcium current responsible for the action potential in intestinal smooth muscle. Its action at this site *in vivo* would be expected to reduce the velocity of action potential upstroke and attenuate action potential discharge and contraction in the intestine. This property might be expected to be useful in conditions such as irritable bowel syndrome which are characterized by hypermotility of the smooth muscle.

References

- AARONSON, P.I., BOLTON, T.B., LANG, R.J. & MACKENZIE, I. (1988). Calcium currents in single isolated smooth muscle cells from the rabbit ear artery in normal-calcium and high-barium solutions. *J. Physiol.*, **405**, 57–75.
- BAUMGARTNER, A., DRACK, E., HALTER, F. & SCHEURER, U. (1985). Effects of pinaverium bromide and verapamil on the motility of the rat isolated colon. *Br. J. Pharmacol.*, **86**, 89–94.
- BEAN, P.B. (1984). Nitrendipine block of cardiac calcium channels: High-affinity binding to the inactivated state. *Proc. Natl. Acad. Sci., U.S.A.*, **81**, 6388–6392.
- BEAN, P.B., STUREK, M., PUGA, A. & HERMSMEYER, K. (1986). Calcium channels in muscle cells isolated from rat mesenteric arteries: Modulation by dihydropyridine drugs. *Circ. Res.*, **59**, 229–235.
- BENHAM, C.D., HESS, P. & TSIEN, R.W. (1987). Two types of calcium channels in single smooth muscle cells from rabbit ear artery studied with whole-cell and single-channel recording. *Circ. Res.*, **61**, 1–10–1–16.
- BOLTON, T.B., AARONSON, P.I. & MACKENZIE, I. (1988). Voltage-dependent calcium channel in intestinal and vascular smooth muscle cells. In *Calcium Antagonists, Pharmacology and Clinical Research*. ed. Vanhoutte, P.M., Paoletti, R. & Govani, S. *Ann. N.Y. Acad. Sci.*, **522**, 32–42.
- BRETAUDEAU, J. & FOUSSARD-BLANPIN, O. (1980). Recherche sur le mécanisme d'action du bromure de pinavérium. *J. Pharmacol. (Paris)*, **11**, 233–243.
- BRETAUDEAU, J., FOUSSARD-BLANPIN, O., BARONNET, R., DESPRAIRIES, R. (1975). Etude pharmacodynamique des propriétés spasmolytiques du bromure de pinavérium. *Thérapie*, **30**, 919–930.
- DROOGMANS, G. & CALLEWAERT, G. (1986). Ca^{2+} -channel current and its modification by the dihydropyridine agonist BAY k 8644 in isolated smooth muscle cells. *Pflügers Arch.*, **406**, 259–265.
- DROOGMANS, G., HIMPENS, B. & CASTEELS, R. (1983). Effect of pinaverium bromide on electrical and mechanical activity of smooth muscle cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **323**, 72–77.
- GANITKEVICH, V. YA., SHUBA, M.F. & SMIRNOV, S.V. (1986). Potential-dependent calcium inward current in a single isolated smooth muscle cell of the guinea-pig taenia caeci. *J. Physiol.*, **380**, 1–16.
- GRENIER, J.F., ANGEL, F., SCHANG, J.C., CRENNER, F. & SAID, A. (1983). Action du pinavérium sur la motricité intestinale. Etude chez le chien. *J. Med. Strasbourg*, **14**, 385–388.
- HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, **391**, 85–100.
- HERING, S., BEECH, D.J., BOLTON, T.B. & LIM, S.P. (1988). Action of nifedipine or BAY K 8644 is dependent on calcium channel state in single smooth muscle cells from rabbit artery. *Pflügers Arch.*, **411**, 590–592.
- HERING, S., BOLTON, T.B., BEECH, D.J. & LIM, S.P. (1989). Mechanism of calcium channel block by D600 in single smooth muscle cells from rabbit ear artery. *Circ. Res.*, **64**, 928–936.
- ITOH, Z. & TAKAHASHI, I. (1981). Inhibitory effect of pinaverium bromide on gastrointestinal contractile activity in conscious dogs. *Arzneim. Forsch./Drug Res.*, **31**, 1450–1453.
- MIRONNEAU, J., LALANNE, C., MIRONNEAU, C., SAVINEAU, J-P & LARIE, J-L. (1984). Comparison of pinaverium bromide, manganese chloride and D600 effects on electrical and mechanical activities in rat uterine smooth muscle. *Eur. J. Pharmacol.*, **98**, 99–107.
- OHYA, Y., TERADA, K., KITAMURA, K. & KURIYAMA, H. (1987). D600 blocks the Ca^{2+} channel from the outer surface of smooth muscle cell membrane of the rabbit intestine and portal vein. *Pflügers Arch.*, **408**, 80–82.
- OKABE, K., TERADA, K., KITAMURA, K. & KURIYAMA, H. (1987). Selective and long-lasting inhibitory actions of the dihydropyridine derivative, CV-4093, on calcium currents in smooth muscle cells of the rabbit pulmonary artery. *J. Pharmacol. Exp. Ther.*, **243**, 703–709.
- RONCA-TESTONI, S., HRELIA, S., HAKIM, G., RONCA, G. & ROSSI, C.A. (1985). Interaction of smooth muscle relaxant drugs with calmodulin and cyclic nucleotide phosphodiesterase. *Experientia*, **41**, 75–76.
- TERADA, K., KITAMURA, K. & KURIYAMA, H. (1987a). Blocking actions of Ca^{2+} antagonists on the Ca^{2+} channels in the smooth muscle cell membrane of rabbit small intestine. *Pflügers Arch.*, **408**, 552–557.
- TERADA, K., NAKAO, K., OKABE, K., KITAMURA, K. & KURIYAMA, H. (1987b). Action of the 1,4-dihydropyridine derivative, KW-3049, on the smooth muscle membrane of the rabbit mesenteric artery. *Br. J. Pharmacol.*, **92**, 615–625.
- TERADA, K., OHYA, Y., KITAMURA, K. & KURIYAMA, H. (1987c). Actions of flunarizine, a Ca^{++} antagonist, on ionic currents in fragmented smooth muscle cells of the rabbit small intestine. *J. Pharmacol. Exp. Ther.*, **240**, 978–983.
- WORLEY, J.F., DEITMER, J.W. & NELSON, M.T. (1986). Single nisoldipine-sensitive calcium channels in smooth muscle cells isolated from rabbit mesenteric artery. *Proc. Natl. Acad. Sci., U.S.A.*, **83**, S746–S750.
- WUYTACK, F., DE SCHUTTER, G. & CASTEELS, R. (1985). Action of pinaverium bromide on calmodulin-regulated functions. *Eur. J. Pharmacol.*, **114**, 85–88.
- YATANI, A., SEIDEL, C.L., ALLEN, J. & BROWN, A.M. (1987). Whole-cell and single-channel calcium currents of isolated smooth muscle cells from saphenous vein. *Circ. Res.*, **60**, 523–533.

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