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Inhibition of field stimulation-evoked relaxations in rat oesophageal smooth muscle by the calcium antagonist PN 200-110

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1 The inhibitory effects of the 1,4-dihydropyridine calcium channel antagonist, PN 200-110 (isradipine), on field stimulation-evoked tetrodotoxin (TTX)-sensitive and -insensitive relaxations were studied in rat oesophageal smooth muscle of the tunica muscularis mucosae.

2 The TTX-insensitive relaxation was inhibited by PN 200-110 in a stereoselective manner with the (+)-(S)-isomer displaying a 1000 fold greater inhibitory potency than the (−)-(R) isomer. A similar potency was noted for inhibition of high K^+-evoked contractions.

3 TTX-sensitive relaxations evoked by field stimulation and contractions elicited by the muscarinic cholinoceptor agonist, *cis*-2-methyl-4-dimethylamino-methyl-1,3-dioxolane methiodide (*cis*-dioxolane) were considerably less sensitive to inhibition by PN 200-110, although, again, stereoselectivity for PN 200-110 was apparent.

4 Pretreatment with (+)-(S)-PN 200-110 resulted in a non-competitive displacement of the Ca^{2+} concentration-response curves obtained in the presence of either isotonic 50 mM KCl or *cis*-dioxolane. The effect of K^+ was 10 fold more sensitive than that of *cis*-dioxolane.

5 The potency rank orders for inhibition of TTX-insensitive field stimulation-evoked relaxations and K^+-mediated contractions in a series of calcium channel antagonists were closely correlated; (+)-(S)-PN 200-110 showing highest potency followed by nifedipine, verapamil, diltiazem, (−)-(R)-PN 200-110.

6 It is concluded that TTX-insensitive relaxations are dependent upon an influx of extracellular Ca^{2+} through potential-operated calcium channels.

Introduction

We have previously demonstrated that, in rat oesophageal smooth muscle, electrical field stimulation results in a relaxation response, provided excitatory cholinergic transmission to the muscle is blocked and an appropriate level of tone induced (Bieger & Triggle, 1985). This relaxation has both a tetrodotoxin (TTX)-sensitive and -insensitive component (Akbarali *et al.*, 1986). The latter was blocked by nifedipine and verapamil (Akbarali *et al.*, 1986) suggesting that Ca^{2+} entry via potential-operated channels was linked to the generation of this form of relaxation. In order to corroborate this hypothesis and to determine possible differences between the calcium channels that support contraction or relaxation, we examined the effects of the 1,4-dihydropyridine derivative, PN 200-110. Hof *et al.* (1984) have recently described the stereoselective cardiovascular effects of this compound noting the greater potency of the (+)-isomer. In the present study, the stereoisomers of PN 200-110 were used as pharmacological probes to study calcium channel-mediated mechanical responses in rat oesophageal smooth muscle and specifically to determine if TTX-sensitive and -insensitive relaxations evoked by field stimulation, on the one hand, and contractions induced by K^+ depolarization and muscarinic cholinoceptor stimulation, on the other, were differentially affected.

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Our results not only confirm the expected stereoselectivity of PN 200-110 in a non-vascular smooth muscle preparation, but demonstrate that TTX-insensitive relaxations and depolarization-induced contraction are equally sensitive to inhibition and significantly more sensitive than are cholinoceptor-mediated contractions and TTX-sensitive relaxations.

Methods

Preparation of tissues

Male Sprague-Dawley rats (250–350 g) were killed by a blow on the head and bled through the jugular veins. The oesophagus was dissected out and the tunica muscularis mucosae with the attached submucosal nerve plexus was set up for isometric tension recording after the striated tunica externa was removed by microdissection. The tunica muscularis mucosae (oesophageal smooth muscle) was divided into either three or four segments of 1–1.5 cm length corresponding to proximal, one or two middle segments, and a distal segment. The muscle was mounted on a tissue holder bearing two concentric platinum electrodes spaced 10 mm apart and placed in a 25 ml jacketed organ bath filled with Tyrode solution. The tissues were maintained at a preload of 0.3 g.

Longitudinal isometric tension was recorded by means of a force transducer (Grass FT 03) on a Grass inkwriter. The preparations were aerated with 95% O_2/5% CO_2. The Tyrode solution had the following composition (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.88, NaH_2PO_4 0.36, $NaHCO_3$ 12.0, and glucose 5.5.

Ca^{2+}-free medium was prepared by omitting $CaCl_2$ from the Tyrode buffer. Depolarizing isotonic potassium (50 mM) solution was prepared by equimolar substitution of NaCl with KCl. $CaCl_2$ was omitted from Ca^{2+}-free 50 mM isotonic depolarizing K^+ solution. All solutions were maintained at pH 7.4 by continuous aeration.

The tissues were allowed to equilibrate for 1 h in Tyrode solution containing 1.8 mM Ca^{2+} before the start of each experiment. Calcium antagonists were applied to the bath under subdued light conditions and either before or after tissues were made to generate active tonus. The muscarinic agonist, *cis*-2-methyl - 4 - dimethylamino - methyl -1 , 3 dioxolane methiodide (*cis*-dioxolane) was chosen because of its lack of nicotinic activity, high affinity for muscarinic receptors and resistance to cholinesterases (Chang *et al.*, 1972).

The effective concentration of the muscarinic agonist used was that which allowed maximal amplitude of TTX-insensitive relaxations as previously described (Akbarali *et al.*, 1986). In order to reveal TTX-sensitive relaxations to field stimulations, it was necessary to expose the tissue to a concentration of (−)-hyoscine methylbromide just sufficient to block field stimulation-evoked contractions and next to induce active tone with a concentration of *cis*-dioxolane large enough to overcome the postjunctional cholinoceptor block, but leaving muscle contractions evoked by endogenous release of acetylcholine blocked. In this manner, relaxations due to activation of intramural inhibitory nerves could be detected. We have previously noted that agonists other than muscarinic do not produce a maintained tonus in this tissue, thus requiring this procedure to be followed (Akbarali *et al.*, 1986). Experimental protocols involving the analysis of TTX-insensitive responses were conducted, unless otherwise stated, in the presence of 0.1–0.3 μM TTX.

Procedures for electrical stimulation

The tissues were field-stimulated with rectangular pulses generated by a Grass S88 stimulator. Stimulation parameters (pulse rate, width and amplitude) were: 8 Hz, 0.5 ms and 40 V for eliciting TTX-sensitive responses and 4 Hz, 2 ms, 40 V for TTX-insensitive responses (Akbarali *et al.*, 1986). However, this did not permit an absolute separation of the two types of relaxation since in some tissues TTX-insensitive relaxations were also evident at the short pulse width.

Experimental procedures

Cumulative Ca^{2+} concentration-response curves were constructed by first incubating tissues for at least 30 min in Ca^{2+}-free buffer with repeated washings with the same. Prior to inducing tone with Ca^{2+}, the tissues were exposed for 10 min to either 0.1 μM *cis*-dioxolane or 50 mM K^+ in Ca^{2+}-free buffer. $CaCl_2$ was added cumulatively, in the presence of either *cis*-dioxolane or 50 mM K^+, to achieve bath concentrations of 0.1–1.8 mM.

In preliminary experiments it was established that cumulative Ca^{2+} responses were only reproducible once when separated by a 30 min interval of Ca^{2+}-free washing as depression of the maxima occurred with the third Ca^{2+} exposure. Accordingly, the tissues were first subjected to a control Ca^{2+} challenge followed 30 min later, after repeated washings in Ca^{2+}-free solutions, by the second Ca^{2+} challenge in the presence of the antagonist. In each case, *cis*-dioxolane or K^+ was applied 10 min before addition of Ca^{2+} and remained in the bath during the experiment.

Responses to high K^+ consisted of an initial

phasic and a prolonged tonic rise in tension (Akbarali *et al.*, 1986). In order to determine the inhibitory effect of calcium antagonists, tissues were pretreated for 10 min before the addition, in the presence of the antagonist, of the high K^+ buffer.

Statistical analysis

Results are expressed as mean ± s.e.mean. Student's *t* test was used to assess the significance of differences between two mean values. The Ca^{2+} concentration-response curves were plotted according to the method of Carpenter (1986). Cumulative concentration-response curves were normalised and log concentrations interpolated for 7–8 predetermined response levels. Log concentrations were then averaged and, for each pre-determined response level, a mean log-concentration was obtained and expressed as a percentage of the maximally attainable response. This method was applied in order to preserve the slope of the concentration-response curves (Carpenter, 1986).

Drugs

Drugs were obtained from the following sources: tetrodotoxin, (−)-hyoscine methylbromide (Calbiochem, U.S.A.). *Cis*-dioxolane was generously donated by Dr D.J. Triggle, State University of New York at Buffalo, U.S.A.; the enantiomers of PN 200-110 [isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-5-methoxycarbonyl-2,6-dimethyl-3-pyridinecarboxylate] by Dr R.P. Hof, Sandoz Ltd., Switzerland; nifedipine by Bayer, A.G., Germany; verapamil by Knoll A.G., Germany; diltiazem by Nordic, Canada.

Results

Preferential inhibition of tetrodotoxin-insensitive relaxation and K^+-induced contraction

Electrical field stimulation of rat oesophageal muscularis mucosae elicited a relaxation after excitatory cholinergic responses were blocked with TTX (0.1 μM), and tension induced with the muscarinic agonist *cis*-dioxolane (0.1 μM) as described previously (Bieger & Triggle, 1985). The mean maximal amplitude of TTX-insensitive relaxations obtained in tissues precontracted with *cis*-dioxolane was 0.219 ± 0.013 g ($n = 57$), confirming previous results obtained in muscle pre-contracted with muscarine (Akbarali *et al.*, 1986) and amounting to 9.6–13.2% of the maximal contractions to muscarinic receptor activation of the proximal and distal oesophagus respectively. TTX-insensitive relaxations were inhibited by PN 200–110 in a stereoselective manner (Figures 1 and 2). The (+)-(S)-siomer was considerably more potent than the (−)-(R)-isomer with IC_{50} values of 0.2 nM and 200 nM, respectively. The calcium antagonist produced inhibition of similar magnitude when applied either before or during

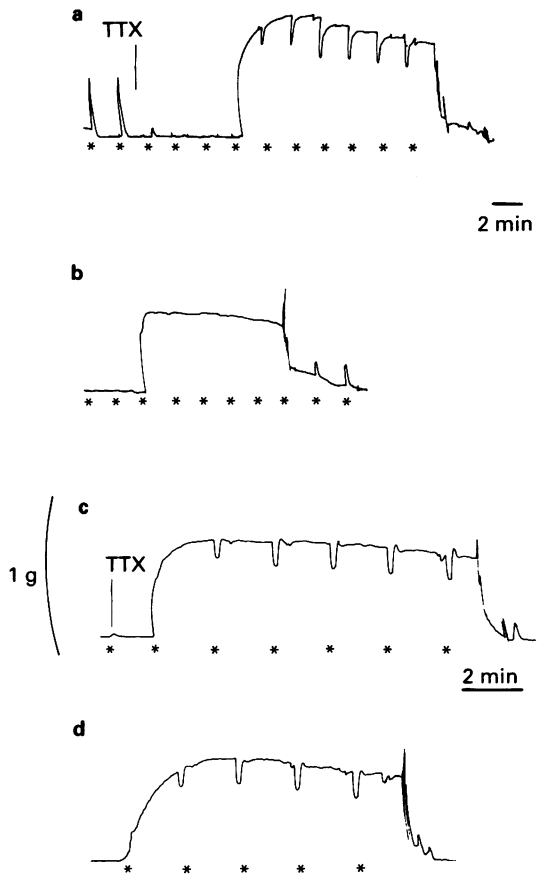

Figure 1 Stereoselective inhibition of field-stimulated relaxation in rat oesophageal tunica muscularis mucosae by the two enantiomers of PN 200-110. Isometric tension records from two isolated oesophageal smooth muscle segments showing effects of (+)-(S)-PN 200-110 (a, b) and (−)-(R)-PN 200-110 (c, d). Control tetrodotoxin (TTX)-insensitive relaxations (a, c) were observed after excitatory responses to field-stimulation were blocked with 0.15 μM TTX and tension induced with 0.1 μM *cis*-dioxolane. After washout with Tyrode (w), the tissues were pretreated with 1 nM (+)-(S)-PN 200-110 (b) or 5 nM (−)-(R)-PN 200-100 (d) for 10 min and re-challenged with 0.15 μM TTX and 0.1 μM *cis*-dioxolane. Note loss of relaxation responses in (b) and persistence in (d). Asterisks at the bottom of each recording indicate 10 s train of field stimulation delivered every 1.5 min at 2 ms pulse width, 4 Hz pulse rate, and 40 V amplitude.

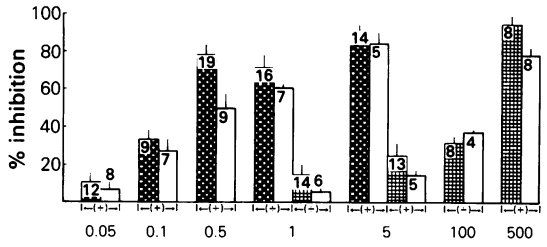

Figure 2 Inhibition of tetrodotoxin (TTX)-insensitive relaxations and K^+ contractions by enantiomers of PN 200-110. The percentage inhibition of TTX-insensitive relaxations was calculated with respect to control responses obtained before pretreatment with PN 200–110. K^+ contractions were produced by isotonic depolarizing 50 nM KCl buffer. Percentage inhibition was determined for the tonic component of the contraction after pretreatment with PN 200-110 for 10 min and continued presence of PN 200–110 during exposure to the K^+ buffer. Cross-hatched columns represent inhibition of TTX-insensitive relaxations and open columns inhibition of K^+. Concentration of PN 200-110 (nM) is indicated below each set of columns. (+) and (−) enantiomers refer to checkered and grid columns, respectively. Number of tissues are indicated within columns.

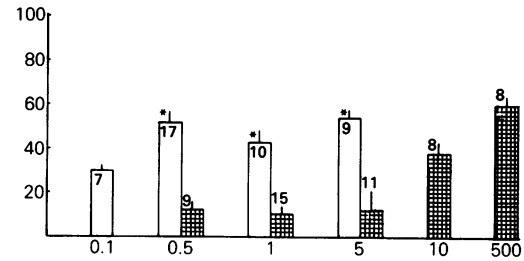

Figure 3 Inhibition of *cis*-dioxolane-induced tone by enantiomers of PN 200-110. Percentage inhibition of 0.1 μM *cis*-dioxolane-induced tone was calculated with respect to control responses. PN 200-110 (nM) concentrations are indicated below columns, % inhibitions on the ordinate scale. Open columns represent (+)-(S)-PN 200-110 and hatched columns (−)-(R)-PN 200-110. Number of tissues are given within the columns. Vertical bars present standard errors. Significant difference $^*P < 0.05$.

steady-state tonus. Thus, pretreatment with (+)-(S)-PN 200-110 1 nM inhibited the response by 65 ± 9% ($n = 10$) and application at steady state tonus by 65 ± 8% ($n = 10$). In the latter case, inhibition reached its maximum in 7–10 min. In tissues contracted with isotonic 50 mM K^+ solution, irrespective of the extracellular Ca^{2+} contraction in the bath and the level of tonus, field stimulation failed to elicit a relaxation. In *cis*-dioxolane-contracted tissues, TTX-insensitive relaxations were, however, evident at low Ca^{2+} concentrations at which tone was not maintained.

As will be noted in Figure 1, the inhibition by PN 200–110 of field stimulation-evoked relaxation of the precontracted tissue was accompanied by a reduction in *cis*-dioxolane-evoked tonus. Therefore, the ability of PN 200-110 to diminish muscle contractility required further analysis. In particular, it appeared worthwhile to compare the antagonism of PN 200-110 against *cis*-dioxolane as opposed to high K^+. As shown in Figures 2 and 3, PN 200-110 displayed a stereoselective antagonism against both stimulants; however, *cis*-dioxolane contractions were clearly less completely blocked than were TTX-insensitive relaxations and K^+ contractions.

When compared with TTX-insensitive relaxations, the TTX-sensitive relaxations displayed a considerably lower sensitivity to PN 200-110 and did not undergo reversal upon restoration of tone (Figure 4). Nonetheless, a reduction in relaxation amplitude occurred (Figure 4c, d) upon restoration of tone. This attenuation of TTX-sensitive responses was evident irrespective of whether or not tissues displayed a TTX-insensitive component at short pulse width (Figure 4, c vs d).

Non-competitive nature of inhibition

In order to clarify the nature of the antagonism by PN 200-110 towards *cis*-dioxolane and K^+ we next examined effects on the concentration-response relationship to Ca^{2+} in tissues preincubated with these two agonists.

As illustrated in Figure 5, the antagonism by (+)-(S)-PN 200-110 was non-competitive towards both *cis*-dioxolane and K^+, resulting in a down- and rightward shift of the Ca^{2+} concentration-response curve. Furthermore, (+)-(S)-PN 200-110 was approximately 10 times more potent in inhibiting Ca^{2+} responses in high K^+ than in inhibiting Ca^{2+} responses mediated by *cis*-dioxolane, confirming results described above. The pD_2 for Ca^{2+} in the presence of *cis*-dioxolane, 0.1 μM, and isotonic K^+, 50 mM, was 0.74 ± 0.03 mM ($n = 20$) and 1.31 ± 0.06 mM ($n = 6$), respectively.

The relative magnitude of depression of K^+ responses in the presence of PN 200-110 was similar to the extent of inhibition of the TTX-insensitive relaxtions. Thus, at 0.1 nM (+)-(S)-PN 200-110, the maximal response to K^+ was depressed by 34 ± 12% and TTX-insensitive relaxations by 34 ± 6%. At 0.5 nM, the *cis*-dioxolane response was reduced by 18 ± 7%, and K^+ contraction and TTX-insensitive relaxations were inhibited by 82 ± 4% and 79 ± 5%, respectively.

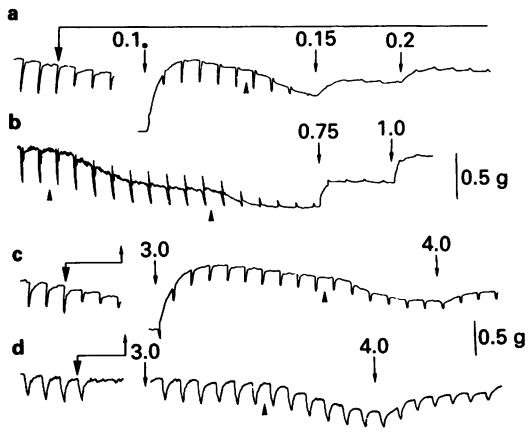

Figure 4 Selective blockade of tetrodotoxin (TTX)-insensitive field-stimulated relaxation by (+)-(S)-PN 200-110. Traces were recorded from four different preparations (3 rats) corresponding to middle thoracic (a, b) and distal (c, d) segments of oesophageal smooth muscle. Contractile responses to field stimulation were eliminated by incubating the tissues with 0.25 μM TTX (a) and/or 5 nM hyoscine methylbromide (a–d), and tonus was induced with *cis*-dioxolane. Bath application of agents was as follows: TTX, 0.25 μM (↓); (+)-(S)-PN 200-110, 1 nM (ι) and, for initial tone induction in the absence (*), right half of (a), or in the presence of hyoscine methylbromide (b–d), *cis*-dioxolane was administered cumulatively in μM concentrations, as indicated (↓). Field stimulation-evoked relaxation in the presence of TTX is blocked by PN 200-110, 1 nM (a) and 2 nM (b) and is replaced by a small contraction when tone is restored with *cis*-dioxolane (a, b). Relaxations evoked by field stimulation in the absence of TTX and the presence of hyoscine methylbromide (c, d) were partially resistant to PN 200-110 and not reversed when tone was restored. Field stimulation was delivered in trains of 10 s duration repeated at 1.5 min intervals and consisting of 40 V rectangular pulses. Frequency and pulse width were as follows: short pulse stimulation at 0.5 ms at 8 Hz was used in (a), left half, and (c) and (d). Long pulse stimulation at 4 Hz, 1 ms, right half of (a), or 2 ms in (b). Calibration, as indicated, is the same for (a), (c) and (d).

Nifedipine, verapamil and diltiazem

In normal Tyrode buffer, nifedipine, verapamil and diltiazem inhibited TTX-insensitve relaxations and K^+-induced contractions more potently and effectively than *cis*-dioxolane-induced contractions. A correlative plot of IC_{50} values for inhibition of K^+-induced contraction and TTX-insensitive relaxations revealed a slope not significantly different from unity (1.11 + 0.19) (Figure 6).

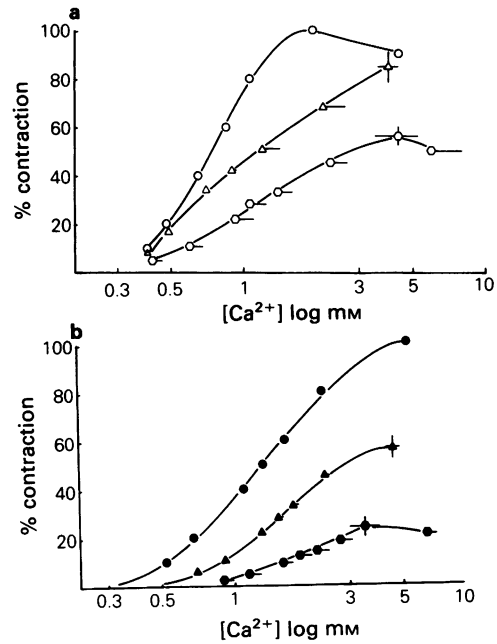

Figure 5 Effects of (+)-(S)-PN 200-110 on the Ca^{2+} concentration-response curves in presence of 0.1 μM *cis*-dioxolane (a) and 50 mM K^+ (b). Curves were plotted as detailed in methods. Horizontal lines represent standard errors. Note approximately equal depression of maxima by 1 nM PN 200-110 in (a) and 0.1 nM PN 200-110 in (b). Maximum responses to 50 mM K^+ were approximately one half (0.73–1.18 g tension) of maximum responses to muscarinic stimulation (1.66–2.27 g tension). In (a), (○) control; (△) 0.5 nM and (○) 1 nM PN 200-110; in (b) (●) control; (▲) 0.1 nM and (●) 0.5 nM.

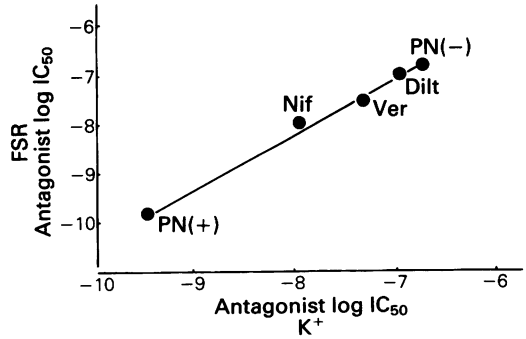

Figure 6 Correlation between inhibition of tetrodotoxin (TTX)-insensitive relaxations and K^+. IC_{50} values are plotted for inhibition of TTX-insensitive relaxations and K^+-induced tone. PN(+) = (+)-(S)-PN 200-110; PN(−) = (−)-(R)-PN 200-110; Nif = nifedipine; Ver = verapamil; Dilt = diltiazem.

Discussion

The present data provide further support for the hypothesis that TTX-insensitive relaxations in rat oesophageal smooth muscle are dependent upon Ca^{2+} entry through potential-dependent channels. The stereoselective inhibition by PN 200-110 is in agreement with other reports (Hof *et al.*, 1984) in that the (+)-(S)-isomer was shown to be more potent than the (−)-(R)-isomer. The calcium channels postulated to be responsible for initiating the TTX-insensitive responses can at present not be distinguished from those required for K^+-induced contractions. This is demonstrated by the equal inhibitory potency of the calcium antagonists with respect to TTX-insensitive relaxations and K^+. In contrast, TTX-sensitive relaxations, presumably resulting from the activation of inhibitory nerves, and cholinoceptor-mediated contractions were comparatively resistant to inhibition by PN 200-110.

Four possible explanations may be considered to account for the paradoxical effects of the calcium antagonist in inhibiting TTX-insensitive relaxations. First, calcium antagonists may act to inhibit release of a mediator from nerve endings. Second, they may act directly on the smooth muscle itself, or, third, on an unidentified interstitial cell type. Fourth, calcium antagonists may have effects unrelated to their actions as calcium entry antagonists. As regards the first two explanations, we have recently demonstrated that TTX-insensitive relaxations most likely result from direct stimulation of the muscle (Akbarali *et al.*, 1987). Thus the oesophageal smooth muscle, cold-stored at 4°C for a minimum of 48 h, was incapable of generating TTX- or atropine-sensitive contractions to field-stimulation but continued to generate relaxations resistant to TTX. Although TTX-insensitive relaxations did decrease with increasing duration of cold-storage this could be correlated with a non-specific impairment of mechanical responsiveness. Moreover, the antagonism of the TTX-insensitive responses produced by low concentrations of (+)-(S)-PN 200-110, combined with its relative lack of effect on the TTX-sensitive relaxations and cholinergic nerve-mediated field-stimulated contractions, reinforces the above inference (Akbarali *et al.*, 1986). Furthermore, we have found that the reproducible demonstration of TTX-sensitive relaxation responses to electrical field stimulation requires an efficient and rapid dissection technique, indicating that the inhibitory fibres mediating TTX-sensitive tissue response are more susceptible to injury and anoxic damage than are the fibres responsible for the contractile events. The fourth possibility is unlikely in view of the subnanomolar concentrations of antagonist required for inhibition, and the demonstrated stereoselectivity and parallelism with the antagonism to K^+ depolarization. Barring an effect on some other as yet unidentified interstitial cell type (analogous to Cajal's interstitial cell; Daniel & Posey-Daniel, 1984), a direct myotropic effect would seem the most plausible explanation.

Consequently, we can return to the issue of whether calcium channels implicated in TTX-insensitive relaxations can be distinguished from those mediating contractile responses. In the case of the contractile response, two main mechanisms could be involved, one requiring opening of potential-operated calcium channels, the other of receptor-operated processes leading to an elevation of intracellular Ca^{2+}. In keeping with findings reported by other workers (see Cauvin *et al.*, 1983), the response to high K^+ (presumably mediated by calcium channels) was observed to be preferentially inhibited by calcium channel antagonists. Thus, it is noteworthy that TTX-insensitive relaxations exhibited the same pharmacological profile as a Ca^{2+} entry process via a potential-operated channel. In contrast, contractions mediated by activation of muscarinic receptors were significantly less sensitive to calcium channel antagonists, suggesting that potential-operated channels are not responsible for all of the maintained tonic phase of the *cis*-dioxolane response.

TTX-insensitive relaxations evoked by field stimulation could not be produced in depolarizing K^+ solution even when extracellular levels of Ca^{2+} were kept low. It has been reported in various smooth muscle preparations that K^+ stimulation allows a sustained intracellular Ca^{2+} level as detected by aequorin and quin-2 whereas the Ca^{2+} signal associated with agonist stimulation is only transient (Morgan & Morgan, 1984; Himpens & Casteels, 1987). It is possible that in the presence of a sustained level of intracellular Ca^{2+} initiated by K^+ stimulation of oesophageal smooth muscle, TTX-insensitive relaxations cannot be observed either due to the depolarized state of the tissue, where membrane potential (E_m) can be assumed to be close to the K^+ equilibrium potential (E_k), or due to the altered gating properties of potassium channels in the membrane (see following paper).

In the same context, the question arises as to whether cholinoceptor activation depolarizes the oesophageal smooth muscle or not. Our evidence at present is indirect; however, we note the failure to observe TTX-insensitive relaxations to occur under high K^+ depolarizing conditions or near maximal cholinoceptor activation which arguably may also result in depolarization (Akbarali *et al.*, 1986). Furthermore, we have previously shown (Ohia *et al.*, 1988) that, although both high K^+ and high *cis*-dioxolane concentrations result in an equivalent increase in ^{45}Ca content of oesophageal smooth

muscle, that induced by K^+ is approximately $\times 100$ more sensitive to inhibition by PN 200-110. This latter result again favours the hypothesized involvement of potential-operated channels in the K^+, but not the *cis*-dioxolane response.

In conclusion, the present experiments describe TTX-insensitive relaxations to field stimulation to be sensitive to calcium channel antagonists, suggesting that a Ca^{2+}-activated process is the basis for such relaxations. By inference, the observed inhibition of TTX-sensitive relaxation would not be attributable to inhibition of putative inhibitory transmitter release, but an impairment of a postjunctional membrane response whose ionic mechanism may partially overlap with that underlying the TTX-insensitive relaxation. TTX-insensitive relaxations have been reported in other vascular and non-vascular smooth muscles (Daniel *et al.*, 1979; Ebeigbe *et al.*, 1983; Cole & Marquis, 1985), however, the cellular mechanisms mediating such relaxations have hitherto not been elucidated. Despite the very high sensitivity, *in vitro*, of gastrointestinal smooth muscle to the inhibitory effects of nifedipine (Bolger *et al.*, 1983) and other calcium channel antagonists, it is noteworthy that the apparent incidence of vascular side effects associated with the clinical use of nifedipine is greater than that reported for gastrointestinal related problems (Sorkin *et al.*, 1985). This may simply reflect that calcium antagonists are primarily used for the treatment of cardiovascular disorders. However, a greater awareness of other potential sites of action of these drugs may improve our understanding of their adverse effects seen in clinical usage. It is thus of interest to note that diltiazem has been reported to have a potential role in the treatment of oesophageal hyper-motility, but not in achalasia (Silverstein *et al.*, 1982).

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