Differential inhibition in the human vas deferens by phenoxybenzamine: a possible mechanism for its contraceptive action

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The effect of phenoxybenzamine on *in vitro* contractile responses of human vasectomy tissues was investigated. Phenoxybenzamine ($0.01\text{--}10\ \mu\text{mol l}^{-1}$) produced a differential inhibition of contractions induced by noradrenaline ($1\text{--}300\ \mu\text{mol l}^{-1}$) in the human vas deferens in that it blocked the longitudinal but not the circular muscle. Contractions of both muscle types induced by noradrenaline ($100\ \mu\text{mol l}^{-1}$) were inhibited by either prazosin ($0.01\text{--}10\ \mu\text{mol l}^{-1}$), a competitive α_1 -selective adrenoceptor antagonist or benextramine ($0.01\text{--}10\ \mu\text{mol l}^{-1}$), an irreversible α_1 -adrenoceptor blocker, but were unaffected by chloroethylclonidine ($0.1\text{--}100\ \mu\text{mol l}^{-1}$), an irreversible α_{1B} -adrenoceptor blocker. In calcium-free/EGTA ($1\ \text{mmol l}^{-1}$) medium, contractions of the longitudinal but not circular muscle induced by noradrenaline ($100\ \mu\text{mol l}^{-1}$) were inhibited by phenoxybenzamine ($0.01\text{--}1\ \mu\text{mol l}^{-1}$). Chloroethylclonidine ($10\text{--}100\ \mu\text{mol l}^{-1}$) was ineffective on both muscle types in calcium-free medium, but, on readmission of calcium, it markedly inhibited the recovery of longitudinal but not the circular muscle. These results suggest a mechanism for the male contraceptive action of phenoxybenzamine by a selective blockade of longitudinal but not circular muscle contractions. The results are discussed in relation to (i) the possible involvement of receptor reserves for noradrenaline and of α_1 -adrenoceptor subtypes coupled in the longitudinal and circular muscle to different mechanisms for increasing cytosolic calcium, (ii) a role in the longitudinal muscle for chloroethylclonidine-sensitive α_1 -adrenoceptors in replenishment of a functional pool of intracellular calcium and (iii) the inhibition of sperm emission by phenoxybenzamine.

Introduction

Phenoxybenzamine inhibits sperm emission in patients without affecting libido, penile erection, orgasm or frequency of coitus (Kedia and Persky, 1981). This effect is reversed on its withdrawal. Its use for the control of premature ejaculation and as a male contraceptive drug is also reported not to affect testicular function, hormonal balance or blood pressure (Homonnai *et al.*, 1984). No evidence of retrograde ejaculation was found.

Phenoxybenzamine is an irreversible α -adrenoceptor antagonist that is more potent at blocking α_1 - than α_2 -adrenoceptors in *in vivo* and *in vitro* preparations (Hamilton *et al.*, 1983; Minneman, 1983). Although its therapeutic action is due mainly to the blockade of α_1 -adrenoceptors, the clinical use of other traditional α_1 -adrenoceptor antagonists are rarely reported to inhibit sperm emission (Smith and Harclerode, 1985; van Zwieten, 1990; Brock and Lee, 1993). Thus, the mechanism underlying the contraceptive action of phenoxybenzamine remains unknown. Thioridazine, a neuroleptic drug with a different therapeutic profile from phenoxybenzamine is also noted for inhibition of sperm emission in patients (Greenberg and Carrillo, 1968; Kotin *et al.*, 1976). An earlier

study (Amobi and Smith, 1992) reports that thioridazine inhibits the contractions induced by noradrenaline or potassium in the longitudinal but not the circular muscle of the human vas deferens. There are no comparable studies on the effects of phenoxybenzamine on the contractions of the human vas deferens.

The present work was undertaken to (i) investigate the effects of phenoxybenzamine on contractions induced by noradrenaline in the longitudinal and circular muscle of the human vas deferens and (ii) determine whether the responses are modified in a manner comparable to the effects reported for thioridazine. A preliminary report of some of these results has been communicated to the Physiological Society (Amobi and Smith, 1994a, b).

Materials and Methods

Tissue preparation and drugs

Epididymal specimens of human vas deferens (5–10 mm long) were obtained after elective vasectomies of healthy, fertile men. The specimens were suspended horizontally (resting tension, 3–5 mN) in a jacketed Perspex chamber and superfused at $2.5\ \text{ml min}^{-1}$ with Krebs' saline ($35.8\text{--}36^\circ\text{C}$),

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gassed with 95% O₂ and 5% CO₂ as described by Amobi and Smith (1992). Mechanical responses were recorded via a force-displacement transducer (compliance 0.4 mm g⁻¹), coupled to a Venture Servoscribe potentiometric recorder. Drugs used were phenoxybenzamine hydrochloride (Smith, Kline and French, Welwyn Garden City), chloroethylclonidine (Research Biochemicals International, Natick, MA), prazosin hydrochloride (Pfizer Ltd, Sandwich), propranolol hydrochloride (ICI Pharmaceuticals, Macclesfield), L-noradrenaline acid tartrate (Winthrop, Guildford), oestradiol (BDH Chemicals, Poole), desipramine (Geigy Pharmaceuticals, Horsham) and from Sigma (Poole): benextramine, L-ascorbic acid and EGTA. The perfusates also contained ascorbic acid (20 µmol l⁻¹) as an antioxidant; oestradiol (1 µmol l⁻¹) and desipramine (0.1 µmol l⁻¹) as inhibitors of extraneuronal and neuronal noradrenaline uptake, respectively, and the β-adrenoceptor blocker, propranolol (1 µmol l⁻¹). Calcium-free solution was prepared by isotonic replacement of CaCl₂ with NaCl and the addition of EGTA (1 mmol l⁻¹).

Contractile responses of human vas deferens specimens

Different vasectomy specimens exhibited different patterns of mechanical response (Amobi and Smith, 1992, 1993a). Of 190 specimens stimulated with noradrenaline (100 µmol l⁻¹), 81 showed only increases in tension (i.e., shortening responses), 89 displayed decreases from and recovery to resting tension (i.e., lengthening responses) and 20 showed an initial increase in tension followed by a decrease below and recovery to resting tension. Responses from the right and left vas deferens of the same patient were not always similar. Previous histological studies have revealed a predominance of either longitudinal or circular muscle in specimens that displayed, respectively, shortening or lengthening. This finding, in conjunction with measurements of changes in luminal pressure, have identified the tissue shortening as being due to contraction of the longitudinal muscle and the tissue lengthening as resulting from the active contraction of the circular muscle (Anton and McGrath, 1977; Smith and Bray, 1990; Amobi and Smith, 1993b, 1995). The quantitative measurements described in the present study were from specimens that displayed either only shortening (i.e., contractions of the longitudinal muscle) or only lengthening (i.e., circular muscle responses). However, even with these, there is presumably some degree of mixing of the muscle types (Amobi and Smith, 1992).

Experimental procedures and analysis

Dose-response curves to noradrenaline were obtained using a progressive but non-cumulative dose sequence with exposures of 5–7 min at intervals of 10–12 min. The contractile response of either the longitudinal or circular muscle was measured as the maximum sustained change from passive resting tension (i.e., tonic responses) in the presence of noradrenaline. Rhythmic activity was excluded. A sigmoid curve fitting computer program was used to analyse the dose-response curves and to determine the concentrations of noradrenaline producing 50% of the maximum response (EC₅₀)

before exposure to phenoxybenzamine. Results are given as means ± SEM and *n* refers to the number of preparations. Only one preparation was obtained from each specimen. Statistical analysis was carried out using Student's *t* test and, where appropriate, a one-way analysis of variance (ANOVA). A significant *F* value from ANOVA tests with *P* < 0.05 was followed by comparison of means using Tukey's honestly significant difference. Differences between the mean of control and experimental groups were considered significant at *P* < 0.05 (*) and highly significant at *P* < 0.01 (**).

Results

Effects of phenoxybenzamine

In drug-free medium, the longitudinal and circular muscle showed a comparable sensitivity to noradrenaline with EC₅₀ values of 20.6 ± 5.3 µmol l⁻¹ (*n* = 6) and 22.6 ± 8.7 µmol l⁻¹ (*n* = 5). However, phenoxybenzamine produced different patterns of inhibition in the muscle types (Fig. 1). In the longitudinal muscle (Fig. 1a), phenoxybenzamine (0.1 µmol l⁻¹ applied for 15 or 25 min or 1.0 µmol l⁻¹ for 15 min) inhibited the contractions and progressively reduced the maximum response. In contrast, contractions of the circular muscle were much less susceptible to phenoxybenzamine (Fig. 1b). Phenoxybenzamine (0.1 or 1.0 µmol l⁻¹ applied for 15 min) inhibited contractions induced by low doses of noradrenaline (< 10 µmol l⁻¹) without affecting the maximum response. An increase in the duration of exposure (0.1 µmol l⁻¹ applied for 25 min) slightly (*P* > 0.2) depressed (by 19%) the maximum response.

Similar results were observed when the antagonist was applied simultaneously in the bath and through the lumen of the tissues to enhance access of the drug. Phenoxybenzamine (0.1 µmol l⁻¹ applied for 15 min) reduced the maximum contraction of only the longitudinal (by 54.5 ± 6.6%; *n* = 4) but not the circular muscle (*n* = 6). A tenfold increase in the concentration of phenoxybenzamine (1.0 µmol l⁻¹) depressed the maximum contraction of the longitudinal muscle by 67 ± 9.7% (*n* = 4) and the circular muscle by 28.1 ± 7.1% (*n* = 6).

Effects of prolonged exposure to phenoxybenzamine, benextramine or prazosin

Contractions of the longitudinal and circular muscle to maximal effective concentrations of noradrenaline (100 µmol l⁻¹) were studied after prolonged (35–40 min) superfusion with phenoxybenzamine; benextramine, an irreversible α₁-adrenoceptor antagonist with a different mechanism of action (Benfy, 1982), or prazosin, a competitive α₁-adrenoceptor antagonist.

Benextramine (0.01–10 µmol l⁻¹) or prazosin (0.01–10 µmol l⁻¹) inhibited contractions of both longitudinal and circular muscle (Fig. 2). In contrast, phenoxybenzamine inhibited contractions of only the longitudinal (Fig. 2a) but not the circular muscle (Fig. 2b) except at 10 µmol l⁻¹. A similar effect was observed when graded concentrations of

Fig. 1. Effects of pretreatment with phenoxybenzamine on contractions of human vas deferens in response to noradrenaline (applied in the bath for 5–7 min; (a) longitudinal; (b) circular muscle). Dose-response curves were determined in the absence ($\circ$; controls; $n = 5-6$) and after pretreatment with phenoxybenzamine applied in the bath for different durations: $0.1 \mu\text{mol l}^{-1}$ either for 15 min ($\bullet$; $n = 6$) or 25 min ($\blacktriangle$; $n = 5-7$) and $1.0 \mu\text{mol l}^{-1}$ for 15 min ($\blacksquare$; $n = 5-7$). Tissues were superfused with drug-free Krebs' solution for 35–45 min after the pretreatment. Contractions of the circular compared with the longitudinal muscle were much less sensitive to inhibition by phenoxybenzamine. Data are expressed as a percentage of the maximum response corrected for time-dependent change in tissue sensitivity to noradrenaline. Maximum control responses were $0.74 \pm 0.09 \text{ mN}$ ($n = 24$; longitudinal muscle) and $0.31 \pm 0.03 \text{ mN}$ ($n = 23$; circular muscle). All values represent means $\pm$ SEM.

phenoxybenzamine were applied simultaneously in the bath and via the tissue lumen ($n = 5$, not shown).

These remarkably different effects on the muscle types are also observed clearly in specimens that displayed a combined response of the longitudinal (upward contractions) and circular (downward contractions) muscle to noradrenaline ($100 \mu\text{mol l}^{-1}$; Fig. 3). Prazosin ($0.1-10 \mu\text{mol l}^{-1}$, applied only in the bath) inhibited and at $1 \mu\text{mol l}^{-1}$ abolished the contraction of both muscle types. In contrast, phenoxybenzamine ($0.1-10 \mu\text{mol l}^{-1}$, applied only in the bath) inhibited contractions of the longitudinal muscle but appeared to enhance

Fig. 2. Comparative effects of graded concentrations of different α_1 -adrenoceptor antagonists on sequential contractions evoked by noradrenaline ($100 \mu\text{mol l}^{-1}$; applied for 5–7 min) in (a) longitudinal muscle and (b) circular muscle of human vas deferens. Contractions of both muscle types ($\circ$; time-matched controls; $n = 5-6$) were inhibited by either prazosin ($\diamond$; $0.01-10 \mu\text{mol l}^{-1}$; $n = 5$) or benextramine ($\triangle$; $0.01-10 \mu\text{mol l}^{-1}$; $n = 4$). In comparison, phenoxybenzamine in the dose range $0.01-1 \mu\text{mol l}^{-1}$ ($\square$; $n = 6-7$) caused the inhibition of the longitudinal but not the circular muscle. The tissues were exposed to each concentration of antagonist for 30–35 min before activation with noradrenaline and contractions expressed as a percentage of the initial drug-free control in the same tissue. The initial response in the sequence measured $0.89 \pm 0.1 \text{ mN}$ ($n = 21$; longitudinal muscle) and $0.31 \pm 0.05 \text{ mN}$ ($n = 21$; circular muscle). All values represent means $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$.

contractions of the circular muscle. Presumably inhibition of the longitudinal muscle response allows full expression of the phenoxybenzamine-resistant contraction of the circular muscle.

Fig. 3. Typical records showing different effects of (a) phenoxybenzamine and (b) prazosin on the response of specimens that displayed a combination of longitudinal (upward contractions) and circular (downward contractions) muscle responses to noradrenaline ($100 \mu\text{mol l}^{-1}$; drugs were added in the bath). Prazosin (0.1 – $10.0 \mu\text{mol l}^{-1}$) inhibited the contractions of both the longitudinal and circular muscle. Phenoxybenzamine (0.1 – $10.0 \mu\text{mol l}^{-1}$) differentially inhibited the longitudinal but not the circular muscle. The tissues were exposed to each dose of antagonist for 30–35 min before activation with noradrenaline. Horizontal lines indicate period of drug application. Further preparations with combined responses (phenoxybenzamine, $n = 12$; prazosin, $n = 6$) gave similar results.

Effects of phenoxybenzamine in calcium-free medium

Noradrenaline activates smooth muscle by mobilizing both extra- and intracellular calcium. Experiments to clarify the effects of phenoxybenzamine on the α_1 -adrenoceptors coupled to these different mechanisms for increasing cytosolic calcium were carried out. Thus, contractions to noradrenaline ($100 \mu\text{mol l}^{-1}$) were studied during prolonged perfusion in calcium-free media and on restoration of calcium in the presence and absence of phenoxybenzamine.

The withdrawal of calcium from the perfusing medium for 35–40 min inhibited the rhythmic contractions and reduced markedly the sustained (tonic) contraction of the longitudinal and circular muscle to noradrenaline ($100 \mu\text{mol l}^{-1}$).

Phenoxybenzamine (0.1 – $1 \mu\text{mol l}^{-1}$) reliably inhibited the calcium-free contractions of longitudinal (Fig. 4a) but not circular muscle (Fig. 4b). Furthermore, reperfusion with normal Krebs' saline (calcium: 2.5 mmol l^{-1}) in the continued presence of phenoxybenzamine ($1 \mu\text{mol l}^{-1}$) also produced different effects. Relative to time-matched controls, recovery of the contraction in the circular (Fig. 4b) but not the longitudinal (Fig. 4a) muscle was unchanged when noradrenaline ($100 \mu\text{mol l}^{-1}$) was applied 55–70 min and ≥ 90 min after restoration of extracellular calcium. Thus phenoxybenzamine inhibited contractions of the longitudinal but not the circular muscle to noradrenaline both in the presence or absence of extracellular calcium.

Effects of chloroethylclonidine

The involvement of chloroethylclonidine-sensitive α_{1B} -adrenoceptors was examined on responses of the longitudinal and circular muscle. In normal Krebs' saline (calcium: 2.5 mmol l^{-1} ; Fig. 5a) or calcium-free/EGTA (1 mmol l^{-1} ; Fig. 5b, c) solution, chloroethylclonidine (up to $100 \mu\text{mol l}^{-1}$) produced little change in the response of both muscle types to noradrenaline ($100 \mu\text{mol l}^{-1}$). However, on restoration of calcium for 60–90 min, chloroethylclonidine ($100 \mu\text{mol l}^{-1}$) reliably suppressed the recovery of contraction evoked by noradrenaline ($100 \mu\text{mol l}^{-1}$) in the longitudinal but not the circular muscle (Fig. 5b).

Discussion

The main finding of this study is that pretreatment with phenoxybenzamine readily inhibited contractions induced by noradrenaline in longitudinal muscle of human vas deferens but that contractions of circular muscle were substantially resistant to the antagonist. Activation of the tissue either via the intramural nerves or by exogenous noradrenaline involves mainly postjunctional α_1 -adrenoceptors (Birmingham, 1968; McLeod *et al.*, 1973; Anton and McGrath, 1977; Hedlund *et al.*, 1985). This is consistent with the finding that responses of both muscle types to noradrenaline were inhibited by prazosin. The

Fig. 4. Effects of phenoxybenzamine on contractions of (a) longitudinal and (b) circular muscle in response to noradrenaline ($100 \mu\text{mol l}^{-1}$, applied in the bath for 7–10 min) during prolonged perfusion in calcium-free medium containing EGTA (1 mmol l^{-1}) and on restoration of extracellular calcium (2.5 mmol l^{-1} , indicated by breaks on the graphs and abscissa; (○): time-matched controls; $n = 6-7$). Contraction of the longitudinal but not the circular muscle in calcium-free medium was inhibited by phenoxybenzamine (●; $0.1-1 \mu\text{mol l}^{-1}$, applied in the bath for 35–40 min at each concentration, $n = 6-7$). Continued exposure to phenoxybenzamine ($1 \mu\text{mol l}^{-1}$) during restoration of calcium (2.5 mmol l^{-1} , 55–70 and ≥ 90 min) also produced a differential inhibition of the muscle types. Data are expressed as a percentage of control evoked by noradrenaline ($100 \mu\text{mol l}^{-1}$; longitudinal muscle: $0.51 \pm 0.06 \text{ mN}$, $n = 12$ and circular muscle: $0.45 \pm 11 \text{ mN}$, $n = 14$) before withdrawal of calcium. All values represent means $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$.

relative resistance of circular muscle to phenoxybenzamine could be due to reserve α_1 -adrenoceptors for noradrenaline. However, irreversible inactivation of α_1 -adrenoceptors with benextramine, in comparison with that by phenoxybenzamine, inhibited contractions of both muscle types. Furthermore, a differential inhibition of the muscle types by phenoxybenzamine was also observed in calcium-free solution. In other tissues, contractions evoked in calcium-free media exhibit no receptor reserve for the agonist (Heaslip and Rahwan, 1983; also see Takeyasu *et al.*, 1979; Nichols and Ruffolo, 1988; Suzuki *et al.*, 1990).

Timmermans *et al.* (1983, 1985) reported that phenoxybenzamine but not benextramine selectively inactivates "... α_1 -adrenoceptor-mediated contractions that are not dependent on the influx of extracellular calcium". Our previous studies showed that the muscle types in the human vas deferens rely to different degrees on different mechanisms for increasing cytosolic calcium. Thus, direct activation by depolarization with high concentrations of extracellular potassium more readily evokes tonic contraction of the circular than it does the longitudinal muscle (Smith and Amobi, 1992; Amobi and Smith, 1993a). Caffeine, an agent that contracts smooth muscle by releasing intracellular calcium, predominantly activates longitudinal but rarely circular muscle (Amobi and Smith, 1993a, 1995; see also Foveaux *et al.*, 1987). However, noradrenaline contracted both longitudinal and circular muscle in calcium-free media and thus appears able to release intracellular calcium in both muscle types. This contrasts with the effect of caffeine and suggests that α_1 -adrenoceptors in the longitudinal and circular muscle may be coupled to different mechanisms of intracellular calcium release. Initial pharmacological studies (Han *et al.*, 1987) suggested that responses using intracellular and extracellular calcium depended, respectively, on activation of chloroethylclonidine-sensitive and insensitive α_1 -adrenoceptors, putatively identified as α_{1B} - and α_{1A} -subtypes. There is now evidence that activation of these subtypes is coupled, via different biochemical pathways, to intracellular calcium release mechanisms (see Wilson and Minneman, 1990).

Furukawa *et al.* (1994) reported that responses induced in the human vas deferens by phenylephrine were relatively insensitive to chloroethylclonidine. Presumably, most of this response depends on intracellular mechanisms, as the responses are largely resistant to the calcium entry antagonist, nifedipine. Contractions of the rat vas deferens induced by exogenous noradrenaline show a comparable insensitivity to chloroethylclonidine (Martinotti *et al.*, 1991; Mallard *et al.*, 1992; Ohmura *et al.*, 1992; Aboud *et al.*, 1993; Bultmann *et al.*, 1994). We have reported that contractions of the rat vas deferens in response to noradrenaline in the absence of extracellular calcium are inhibited by the α_{1A} -adrenoceptor subtype selective antagonist, 5-methylurapidil, but not by chloroethylclonidine (Amobi and Smith, 1994b). In the study reported here, chloroethylclonidine-sensitive α_1 -adrenoceptors appear not to be involved directly in activating calcium influx-dependent or influx-independent contractions of either muscle type. However, chloroethylclonidine effectively blocked the recovery of responses from calcium-free conditions in the longitudinal but not in the circular muscle. The report by Schwietert *et al.* (1991) that phenoxybenzamine exhibits a much higher affinity (1000-fold) for a population of α_1 -adrenoceptors inactivated by

chloroethylclonidine suggests a mechanism for the selectivity of phenoxybenzamine: the longitudinal muscle may depend for much of its response on a calcium store which is replenished via activation of chloroethylclonidine-sensitive α_1 -adrenoceptors. Pretreatment with phenoxybenzamine would therefore selectively block the replenishment of the activator pool of calcium in the longitudinal muscle. Presumably, it would be this same pool that is readily activated directly by caffeine, or by α_1 -selective adrenoceptor agonists, phenylephrine or methoxamine (Amobi and Smith, 1993b, 1995; Furukawa *et al.*, 1994) and is sensitive to ryanodine (Amobi and Smith, 1994c).

Functionally, the co-ordinated contractions of longitudinal and circular muscle during neural excitation provides the propulsive component to sperm transport and ensures sperm emission (Batra, 1974). In relation to the contraceptive action of phenoxybenzamine, the physiological consequences of the differential inhibition of longitudinal but not of circular muscle contraction will be, *in vivo*, the disruption of the propulsive function of the vas deferens and a constriction of the tissue lumen. This will impede sperm transport by a sphincter-like effect and inhibit sperm emission. The aspermia and absence of retrograde ejaculation reported in men treated with phenoxybenzamine (Homonnai *et al.*, 1984) does suggest an inhibition of the propulsive component of sperm transport. The effects of phenoxybenzamine reported here are comparable to the actions of the neuroleptic, thioridazine, on the responses of the human vas deferens (Amobi and Smith, 1992). Both drugs, in comparison to their pharmacological and therapeutic counterparts, inhibit sperm emission in patients (Greenberg and Carrillo, 1968; Kotin *et al.*, 1976; Kedia and Persky, 1981; Homonnai *et al.*, 1984). The results of this and previous studies (Amobi and Smith, 1992, 1993a, 1993b, 1994a, 1995) indicate that the muscle types of the human vas deferens differ in physiological and pharmacological properties. Such differences may be exploited in the development of drugs either to enhance the contractile activity of a patent but aperistaltic tissue or to block functional propulsion and provide a short-term, reversible, non-hormonal male contraceptive.

Fig. 5. Effects of chloroethylclonidine on contractions of the longitudinal and circular muscle in response to noradrenaline ($100 \mu\text{mol l}^{-1}$, applied in the bath for 7–10 min). (a) Contractions of the longitudinal ($\circ$; $n = 3$) and circular ($\square$; $n = 3$) muscle in the normal Krebs' solution (calcium, 2.5 mmol l^{-1}) were unaffected by chloroethylclonidine (0.1 – $100 \mu\text{mol l}^{-1}$, applied for 40–45 min at each concentration). Data are expressed as a percentage of time-matched controls (longitudinal muscle: $0.71 \pm 0.34 \text{ mN}$, $n = 6$ and circular muscle: $0.2 \pm 0.05 \text{ mN}$, $n = 5$). (b,c) Contractions of both muscle types during prolonged perfusion in calcium-free media containing EGTA (1 mmol l^{-1} . $\circ$: time-matched controls; $n = 6$ –7) were also unchanged by chloroethylclonidine ($\bullet$; 10 – $100 \mu\text{mol l}^{-1}$ applied for 40–45 min at each concentration, $n = 4$ each). On restoration of extracellular calcium (2.5 mmol l^{-1} for 40–60 and > 90 min, indicated by breaks on the graph and abscissa) recovery of longitudinal but not the circular muscle contraction was inhibited by chloroethylclonidine ($100 \mu\text{mol l}^{-1}$). Data are expressed as a percentage of control response evoked by noradrenaline ($100 \mu\text{M}$; longitudinal muscle: $0.55 \pm 0.09 \text{ mN}$, $n = 10$ and circular muscle: $0.61 \pm 0.14 \text{ mN}$, $n = 10$) before the withdrawal of calcium. All values represent means $\pm$ SEM. $**P < 0.01$.

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