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Phentolamine-induced rhythmic contractions in bladder detrusor muscle of guinea-pig

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1 Phentolamine caused a rhythmic contraction concentration-dependently without affecting resting tone in the detrusor muscle.

2 Prazosin, yohimbine, propranolol, noradrenaline, clonidine or isoprenaline failed to cause the rhythmic contraction. These agents did not modify the response to phentolamine suggesting no involvement of α-or β-adrenoceptors in the response to phentolamine.

3 Chlorpheniramine, cimetidine, methysergide, SK&F 83566, atropine, bretylium, hemicholinium or tetrodotoxin failed to inhibit the response to phentolamine. These results suggest that the effect of phentolamine is not mediated through histaminergic, 5-hydroxytryptaminergic, dopaminergic or cholinergic systems, or through transmitter release from nerve endings.

4 Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), arachidonic acid but not ATP caused rhythmic contractions which resembled the response to phentolamine. Potassium also caused a contraction with increasing resting tone.

5 Following treatment with nifedipine, or incubation in a Ca^{2+}- free medium, the responses to phentolamine, $PGF_{2\alpha}$, arachidonic acid and potassium were markedly inhibited or abolished.

6 Cyclo-oxygenase inhibitors such as indomethacin, aspirin and corticosterone inhibited or abolished the responses to phentolamine and arachidonic acid but did not inhibit the response to $PGF_{2\alpha}$.

7 The results suggest that the phentolamine-induced rhythmic contraction may, at least in part, result from the cyclo-oxygenase metabolite of arachidonic acid in guinea-pig detrusor muscles and a consequent increase in the transmembrane Ca^{2+}-influx.

Introduction

Phentolamine is generally considered to be a competitive α_1- and α_2-adrenoceptor antagonist without any agonist activity. However, it has been suggested that phentolamine is an agonist at a presynaptic-like adrenoceptor in rabbit vas deferens but not in rat vas deferens (Broadhurst *et al.*, 1983). More recently, Angus & Lew (1984) have also suggested that, in rabbits, phentolamine has an agonist-like activity at presynaptic receptors on cardiac sympathetic nerve terminals and on vascular receptors that mediate vasopressor responses.

We have recently found an interesting result, in guinea-pig bladder, in which phentolamine evoked rhythmic contraction in a concentration-dependent manner. Therefore, experiments were undertaken to clarify the possible mode of the excitatory action of phentolamine using the detrustor muscle of the guinea-pig bladder.

Methods

Male guinea-pigs weighing 300 – 400 g were killed by a blow to the back of the head and the abdomen was opened to remove the bladder. The bladder was excised and smooth muscle strips (approximately 2 mm × 6 mm) were dissected from the detrusor portion of the bladder, avoiding trigone and bladder neck. Ligatures were placed on both ends of the strips and one end was attached to a tissue holder and the other to a force-displacement transducer (Grass FT03) connected to a Grass polygraph on which tension changes were recorded. Each of the strips was then placed in a 20 ml tissue bath containing Krebs-Ringer medium of the following composition (mM): NaCl 120.3, $MgSO_4 7H_20$ 1.3, KH_2PO_4 1.2, $CaCl_2$ 1.2, $NaHCO_3$ 24.2 and glucose 5.8. The solution was maintained at 32°C in order to prevent

spontaneous rhythmic contractions (Weetman, 1972) and aerated with 95% O_2 + 5% CO_2 (pH 7.4) throughout the experiment. The resting tension was adjusted to 1.0 g during an equilibration period of 2 h. Ca^{2+}-free medium was prepared by omitting $CaCl_2$ from the solution, and adding EGTA (0.5 mM) to the medium. In the Ca^{2+}-free medium experiment, tissues were incubated in the medium for 30 min (washed every 10 min with Ca^{2+}-free medium) before the application of drugs. The mean amplitude of rhythmic contraction was obtained by the contractions occurring during the last 10 min of the drug contact period.

The following drugs were used: phentolamine mesylate (Ciba-Geigy), arachidonic acid (Sigma), $PGF_{2\alpha}$ (Upjohn), indomethacin (Merk, Sharp & Dome), aspirin (Bristol-Myers), corticosterone (Sigma), noradrenaline bitartrate (Sigma), clonidine hydrochloride (Boehringer Ingelheim), methoxamine hydrochloride (Burroughs Wellcome), isoprenaline sulphate salt (K & K), prazosin hydrochloride (Pfizer), yohimbine hydrochloride (Sigma), propranolol hydrochloride (Ayerst), atropine sulphate (Nutritional Biochem.), cimetidine hydrochloride (SK&F), chlorpheniramine maleate (Sigma), methysergide maleate (Sandoz), SK&F 83566 ($C_{17}H_{18}$ Br NO. HBr, Smith Klein & French), adenosine triphosphate (Sigma), bretylium tosylate (Burroughs Wellcome), hemicholinium (Aldrich), tetrodotoxin (Sankyo), and nifedipine (Pfizer). Yohimbine and indomethacin were dissolved in absolute ethanol. Arachidonic acid and nifedipine were first dissolved in acetone and then diluted with distilled water. Other drugs were dissolved in distilled water. The doses of the drugs were expressed as final bath concentrations.

Results are expressed or plotted as the mean ± s.e. Student's t test was used for statistical analysis, $P < 0.05$ being considered as significant.

Results

Phentolamine (3×10^{-6} M – 1×10^{-4} M) induced a concentration-dependent increase in the rhythmic contraction without significantly affecting the basal tone in detrusor muscle strips of guinea-pigs (Figure 1). However, in the same preparations ($n = 8$), prazosin (10^{-5} M), yohimbine (10^{-5} M), propranolol (10^{-6} M), noradrenaline (10^{-8} M – 10^{-4} M), methoxamine (10^{-8} M – 10^{-4} M), or isoprenaline (10^{-8} M – 10^{-4} M) failed to cause any significant contraction. In addition, α-adrenoceptor agonists such as noradrenaline, methoxamine or clonidine had no apparent effect on the concentration-response curve of phentolamine (3×10^{-6} M – 10^{-4} M) in all preparations ($n = 5$).

After treatment with nifedipine (10^{-6} M) for 10 min, or incubation of the tissue for 30 min, in a Ca^{2+}-free medium containing EGTA (0.5 mM) the contractile response to phentolamine (10^{-6} M – 10^{-4} M) was completely inhibited in all preparations used ($n = 5$) (Figure 1).

On the other hand, chlorpheniramine (10^{-5} M), cimetidine (10^{-4} M), methysergide (10^{-6} M), SK&F 83566 (10^{-5} M; a dopamine receptor antagonist; Berkowitz *et al.*, 1984), atropine (10^{-6} M – 10^{-5} M), bretylium (10^{-6} M), hemicholinium (10^{-5} M) or tetrodotoxin (10^{-6} M) failed to inhibit the response to

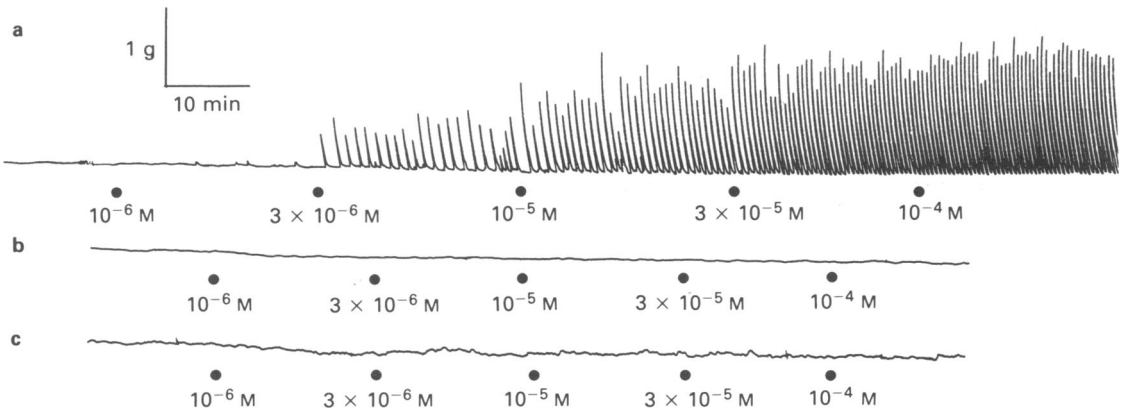

Figure 1 Effect of nifedipine or Ca^{2+}-free medium on the phentolamine-induced rhythmic contraction in guinea-pig detrusor muscles. (a) Phentolamine (10^{-6} M – 10^{-4} M), alone; (b) pretreatment with nifedipine (10^{-6} M) for 20 min; (c) following incubation in a Ca^{2+}-free medium for 20 min. Solid circles indicate the application of phentolamine.

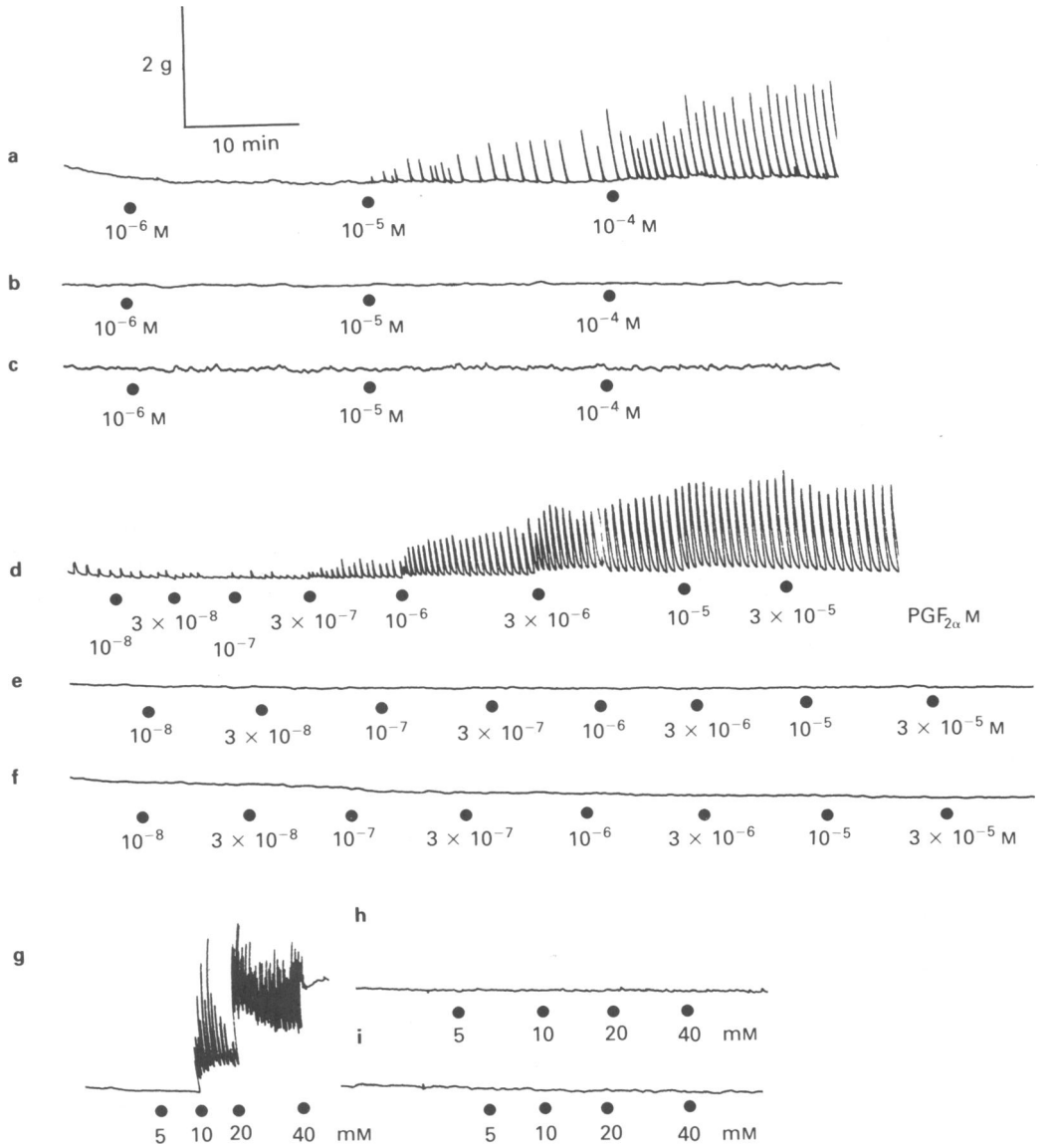

Figure 2 The inhibitory effects of nifedipine and a Ca^{2+}-free medium on the rhythmic contraction induced by archidonic acid (AA), prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) or potassium (K). (a) AA (10^{-6}M – 10^{-4}M), alone; (b) and (c) AA response in the presence of nifedipine (10^{-6}M) or in a Ca^{2+}-free medium respectively; (d) $PGF_{2\alpha}$(10^{-8}M – 3×10^{-5}M), alone; (e) and (f) $PGF_{2\alpha}$response in the presence of nifedipine (10^{-6}M) or in a Ca^{2+}-free medium respectively; (g) K (5 mM – 40 mM), alone; (h) and (i) K response in the presence of nifedipine (10^{-6}M) or in a Ca^{2+}-free medium respectively. Tissues were pretreated with nifedipine for 20 min, or incubated in a Ca^{2+}-free medium for 20 min. Solid circle indicates the application of AA, $PGF_{2\alpha}$ or K.

phentolamine at all concentrations used and these agents, by themselves, did not cause any effect on the detrusor muscle in any of the preparations ($n = 8$).

Treatment with prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$; 10^{-7}M – 10^{-5}M) or arachidonic acid (10^{-5}M – 10^{-4}M) caused rhythmic contractions which were similar to those caused by phentolamine in all preparations ($n = 5$) (Figure 2); these responses were abolished by

nifedipine (10^{-6}M) or by incubation in a Ca^{2+}-free medium containing EGTA (0.5 mM). Potassium (10 mM – 40 mM) caused rhythmic contractions, but also increased the basal tone in all five preparations; again these responses were abolished by nifedipine (10^{-6}M) or by incubation in the Ca^{2+}-free medium containing EGTA (0.5 mM) (Figure 2). Thus the potassium-induced contractions were apparently different from those to phentolamine. In all five experiments, adenosine triphosphate (ATP; 10^{-5}M and 10^{-4}M) caused a single twitch contraction (0.51 ± 0.06 g at 10^{-5}M, 1.1 ± 0.4 g at 10^{-4}M) but failed to cause rhythmic contractions.

Furthermore, 20 min after treatment with indomethacin (10^{-5}M), corticosterone (10^{-4}M) or aspirin (10^{-4}M), the concentration – response curve of phentolamine was significantly shifted to the right from that in untreated control preparations (Figures 3 and 4). Indomethacin at a high concentration (10^{-4}M), abolished the phentolamine response (Figures 3 and 4). Also, indomethacin (10^{-5}M – 10^{-4}M), aspirin (10^{-4}M) and corticosterone (10^{-4}M) inhibited the responses to arachidonic acid (Figures 3 and 5) but had no apparent effect on the responses to $PGF_{2\alpha}$ or potassium (Figure 5) in all six preparations. Further, indomethacin (10^{-4}M) failed to inhibit the responses to ATP (10^{-5}M – 10^{-4}M) ($n = 5$).

Discussion

Phentolamine is generally considered to be a competitive α_1- and α_2-adrenoceptor antagonist without any agonist activity. In the present experiments, however, phentolamine was found to cause a concentration-dependent increase in rhythmic contraction without affecting the basal tone in guinea-pig detrusor muscle. On the other hand, other α-adrenoceptor antagonists (prazosin and yohimbine, respectively) failed to cause any contraction.

Further, the present experiments demonstrated that α-adrenoceptor agonists such as noradrenaline (a mixed adrenoceptor-agonist), methoxamine (an α_1-agonist) and clonidine (an α_2-agonist) did not cause rhythmic contractions or modify the response to phentolamine. A similar lack of effect of α-adrenoceptor antagonists and agonists on the response to phentolamine was observed.

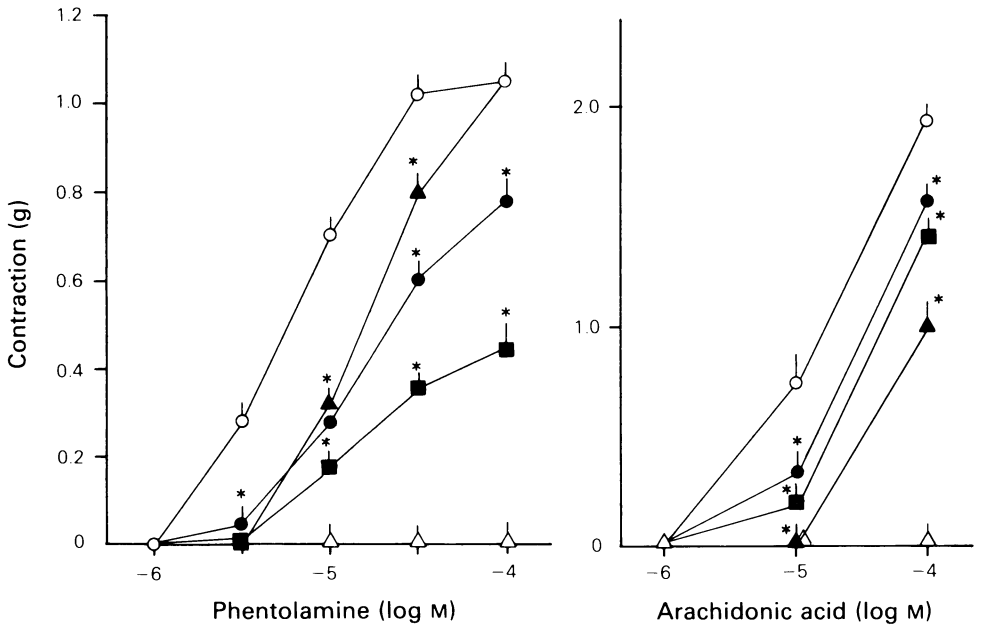

Figure 3 The inhibitory effects of cyclo-oxygenase inhibitors (indomethacin, aspirin or corticosterone) on the concentration-response curves of phentolamine and arachidonic acid in guinea-pig detrusor muscles. Symbols indicate the following: control (○), phentolamine at 10^{-6}M – 10^{-4}M or arachidonic acid at 10^{-6}M – 10^{-4}M, alone; indomethacin at 10^{-6}M (▲) and 10^{-5}M (△); aspirin at 10^{-4}M (■); corticosterone at 10^{-4}M (●). Tissues were pretreated with a cyclo-oygenase inhibitor for 20 min. * Significantly different from control value.

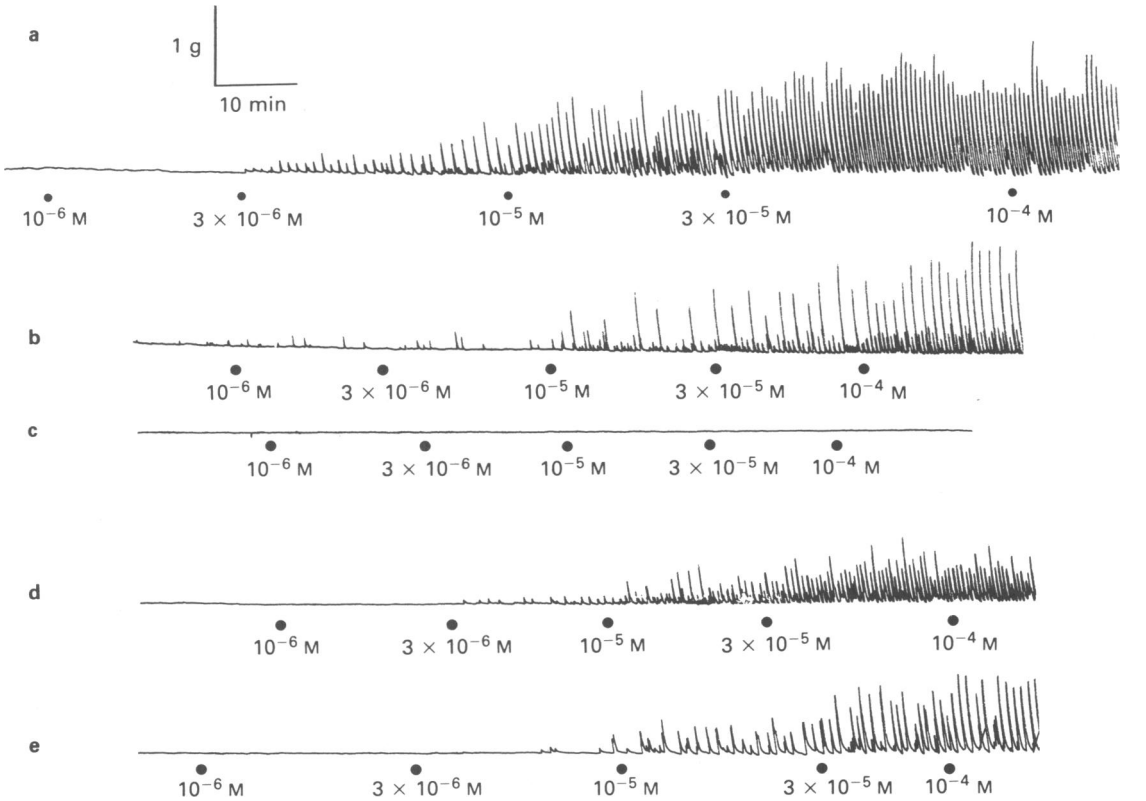

Figure 4 The representative response to phentolamine in the presence of a cyclo-oxgenase inhibitor (indomethacin, aspirin or corticosterone) in guinea-pig detrusor muscles. (a) Phentolamine (10^{-6}M – 10^{-4}M), alone; (b) and (c) in the presence of indomethacin at 10^{-5}M and 10^{-4}M respectively; (d) in the presence of aspirin at 10^{-4}M; (e) in the presence of corticosterone at 10^{-4}M. Tissues were pretreated with a cyclo-oxygenase inhibitor for 20 min. Solid circles indicate application of phentolamine.

It has been previously found that the urinary bladder of guinea-pig fails to show any response to exogenous catecholamines, suggesting a lack of adrenoceptors in guinea-pig bladder (Edvardsen & Setekleiv, 1968). Furthermore, using the same preparation, De Sy (1970) demonstrated that catecholamines, such as noradrenaline, adrenaline and isoprenaline, inhibited the contraction evoked by electrical stimulation and this inhibitory action was blocked by adrenoceptor angonists indicating the presence of only inhibitory α- and β- adrenoceptors.

However, these results suggest that the stimulatory action of phentolamine is not due to the activation of α- or βadrenoceptors. In addition, the effect of phentolamine is not mediated through histaminergic, dopaminergic, 5-hydroxytryptaminergic or cholinergic systems, since none of the antagonists used to inhibit these receptors was effective. Moreover, since inhibitors of adrenergic and cholinergic neurotransmitter release (bretylium and hemicholinium respectively), and tetrodotoxin did not interfere with the response to phentolamine, the effect of phentolamine is not mediated through neurotransmitter release from nerve endings.

In the present experiments, potassium caused a contraction although the configuration of this response was unlike that of the phentolamine-induced contraction. This led us to consider that phentolamine, like potassium, may cause a contraction due to membrane depolarization. However, such a possibility can be eliminated as the phentolamine-induced contraction, but not the potassium-induced contraction, was inhibited by indomethacin.

Several investigators have previously suggested that endogenous prostaglandins play an important role in the maintenance of tone and the development of spontaneous contractility in the mammalian bladder (Eckenfels & Vane, 1972; Bultitude *et al.*, 1976;

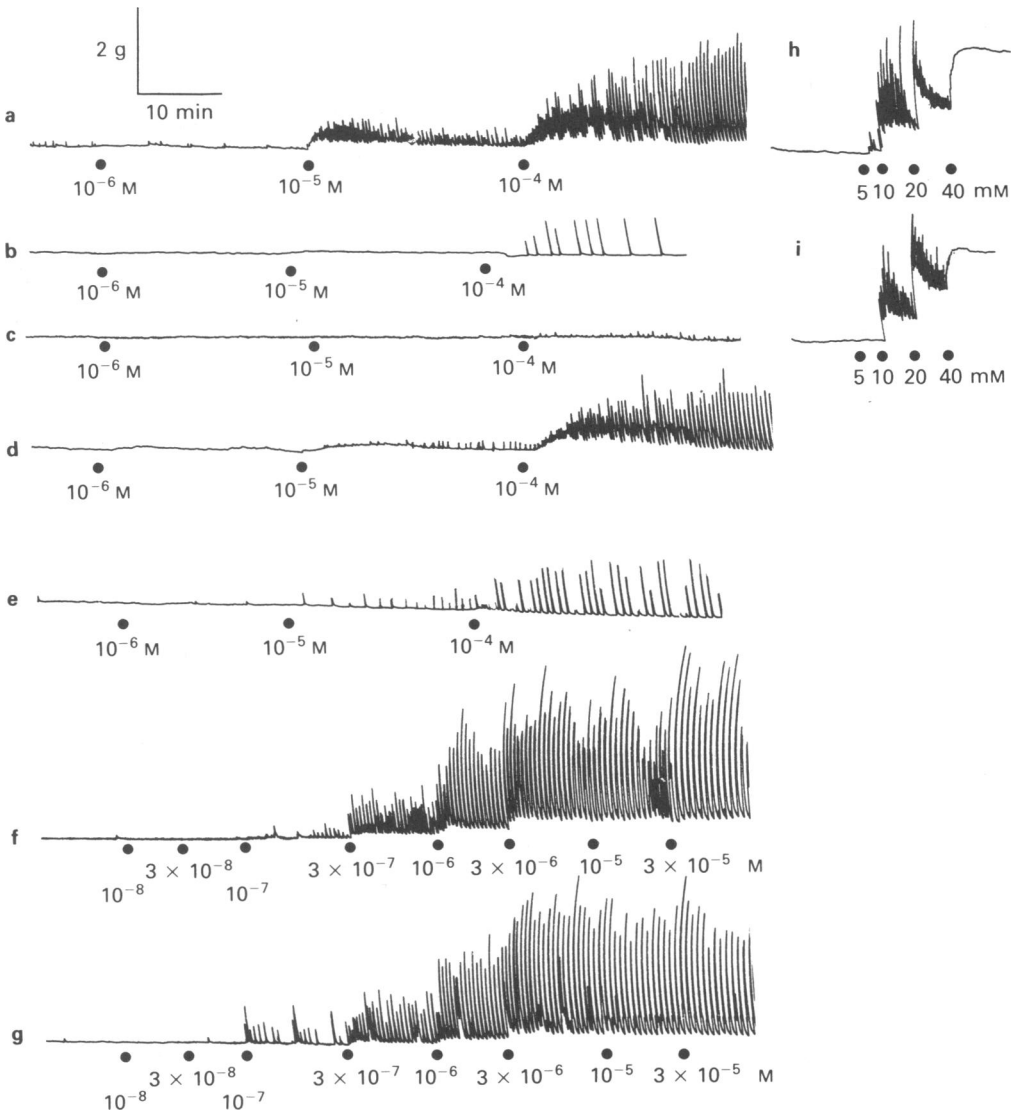

Figure 5 The effects of cyclo-oxgenase inhibitors (indomethacin, aspirin or corticosterone) on the rhythmic contraction induced by arachidonic acid (AA), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and potassium (K) in guinea-pig detrusor muscles. (a) Arachidonic acid (10^{-6}M – 10^{-4}M), alone; (b) and (c) AA response in the presence of indomethacin at 10^{-5}M 10^{-4}M, respectively; (d) AA response in the presence of aspirin at 10^{-4}M; (e) AA response in the presence of corticosterone at 10^{-4}M; (f) $PGF_{2\alpha}$ (10^{-8}M – 3×10^{-5}M), alone; (g) $PGF_{2\alpha}$ response in the presence of indomethacin at 10^{-4}M; (h) K (5 mM – 40 mM), alone; (i) in the presence of indomethacin (10^{-4}M). Tissues were pretreated with the cyclo-oxygenase inhibitor for 20 min. Solid circles indicate the application of AA, $PGF_{2\alpha}$ or K.

Husted *et al.*, 1980). Also, in *vitro*, the prostaglandins induced a contraction of the bladder which was abolished by treatment with indomethacin, a cyclo-oxygenase inhibitor (Bultitude *et al.*, 1976; Anderson & Kohn, 1978; Abrams *et al.*, 1979). In the present experiments, $PGF_{2\alpha}$ and arachidonic acid, a precursor of prostaglandins, caused a rhythmic contraction which was similar to the response to phentolamine, and the effect of arachidonic acid was inhibited by the cyclo-oxygenase inhibitors, in-

domethacin, aspirin and corticosterone. In addition, the cyclo-oxygenase inhibitors also reduced the response to phentolamine in a concentration-dependent manner. These results suggests a possible involvement of endogenous prostaglandins in the response of the guinea-pig bladder to phentolamine.

It is known that transmembrane Ca^{2+}-influx plays an essential role in the maintenance of tone and spontaneous contractility and also in the activation of contractions induced by a variety of stimulants in mammalian and human bladders (Andersson *et al.*, 1980; Maggi *et al.*, 1982; Khanna *et al.*, 1983), since these contractile activities are inhibited by Ca^{2+} entry blockers. In particular, several investigators have demonstrated the inhibitory effect of nifedipine (Ca^{2+}-entry blocker) or Ca^{2+}-free medium on the prostaglandin induced contractions in rabbit and human detrusor muscle (Anderson & Kohn, 1978; Forman *et al.*, 1978). It has been suggested that prostaglandins may serve as regulators of Ca^{2+} binding sites within the cell membrane and thereby modify cell motility (Paton & Daniel, 1967). Anderson & Kohn (1978) suggested that prostaglandins may act at Ca^{2+} channels to augment the movement of Ca^{2-} through membranes or that they might serve a role as carrier for Ca^{2+} in the rabbit detrusor muscle cells.

The present experiments also indicate that nifedipine, similar to Ca^{2+} withdrawal from the medium, abolishes the rhythmic contraction induced by phentolamine as well as the contraction induced by $PGF_{2\alpha}$ or arachidonic acid; whereas tetrodotoxin, which inhibits the fast Na^+ influx, failed to inhibit thse responses.

Therefore, the present experiments suggest that the transmembrane Ca^{2+}-influx, but not the fast Na^+-influx, plays an essential role in the development of the phentolamine-induced rhythmic contraction, which may be mediated through the production of a cyclo-oxygenase metabolite of arachidonic acid, in guinea-pig detrusor muscle. However, further investigation will be required to clarify the precise mechanism of the excitatory action of phentolamine.

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