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Dopamine depresses non-adrenergic, non-cholinergic neurotransmission in the rat bladder

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Abstract—The effects of dopamine on non-adrenergic, non-cholinergic (NANC) neurotransmission have been investigated using rat bladder strips in-vitro. Dopamine administered alone did not produce any effect on the bladder but it depressed responses to NANC nerve stimulation in a dose-related fashion. All doses of dopamine employed depressed KCl-evoked contractions of the bladder to similar extents. The depressant action of dopamine on NANC transmission was not mediated by blockade of purinergic receptors, but was partially reversed by haloperidol. It is suggested that depression of NANC neurotransmission induced by dopamine in the rat bladder is partly mediated by dopaminergic receptors.

Dopamine displays agonist activity at several sites in the central nervous system (Rang & Dale 1991) and peripherally (Goldberg 1972). The peripheral mechanisms of action of dopamine include direct stimulation of cardiac β-adrenoceptors, α-adrenoceptor stimulation by direct activation, or via noradrenaline release, stimulation of dopamine (D_1) receptors on renal vessels and stimulation of prejunctional D_2 receptors on sympathetic nerve endings (Farmer 1966; Goldberg 1972; Makabali et al 1982; Brown et al 1985; Mitchell et al 1987). Recently, a third component of the autonomic nervous system that is non-adrenergic, non-cholinergic (NANC) has been identified. One of the putative transmitter substances suggested for this system is adenosine 5′-triphosphate (ATP) or a related purine compound; hence these nerves have been termed 'purinergic' (Burnstock et al 1970, 1972; Burnstock 1972). It is not yet known whether dopamine influences NANC transmission. This possibility has been examined in the present study using the rat bladder which is innervated by NANC excitatory nerves (Dumsday 1971; Burnstock et al 1972; Brown et al 1979; Lot & Bennett 1982).

Materials and methods

Adult male Wistar rats, 240–280 g, obtained from the National Veterinary Research Institute, Vom, Nigeria were used.

Organ bath studies. Rats were killed by stunning and bleeding, the bladder quickly dissected out and cut open. A portion 3 mm wide and 7 mm long taken from the bladder was threaded at both ends and set up in a 20 mL organ bath as previously described (Lot & Bennett 1982). One end was tied to a pin held in a perspex block and suspended in the organ bath, while the other end was tied to an isometric transducer connected to a Grass polygraph recorder (Model 7D).

The organ bath contained 20 mL of freshly prepared physiological saline (composition (mM): NaCl 118·0, KCl 4·7, $MgSO_4$ 2·5, NaH_2PO_4 1·0, $NaHCO_3$ 30·0, glucose 11·1, $CaCl_2$ 2·5) gassed with 95% O_2–5% CO_2. The bath was maintained at 37 ± 1°C by means of a water jacket connected to a thermostatically controlled pump (Haake). Tissues were allowed 30 min to equilibrate before experiments were started.

Parallel platinum wire electrodes were arranged on either side of the bladder strip and connected to a constant voltage square-wave stimulator (Grass S88 stimulator). Electrical stimuli were delivered as pulses of 140 V and 0·2 ms duration for 10 s every 4 min. The frequency of stimulation was varied as desired.

Drug solutions were freshly prepared in concentrations such that the addition of 0·2 mL gave the final bath concentration (M). The drug contact time was 1 min with an interval between successive doses of at least 5 min. Concentration-response curves were established by graded increases in the concentrations of agonist drugs, frequency-response curves by graded increases in frequency of stimulation. Antagonists and dopamine were always applied 5 min before the agonist drugs or before electrical stimulation.

Drugs. Drugs used were adenosine 5′-triphosphate (Sigma, Poole, Dorset), atropine sulphate (Sigma), haloperidol (Searle, Munich), prazosin hydrochloride (Sigma), propranolol hydrochloride (ICI, UK).

Statistical analysis. Regression lines with confidence limits were calculated for the linear portions of log concentration-response or log frequency-response curves. The significance of differences in slope was used as a measure of concentration or frequency differences as described by Birmingham et al (1970). Maximum responses were compared using Student's unpaired *t*-test.

Results

The parameters used in the present study for field stimulation (140 V, 0·2 ms duration for 10 s every 4 min) have been shown to selectively stimulate nerves of the rat bladder (Lot & Bennett 1982). Field stimulation contracted the rat bladder in a frequency-dependent manner; the log frequency-response curve was shifted ($P<0·05$) to the right with a depressed ($P<0·01$)

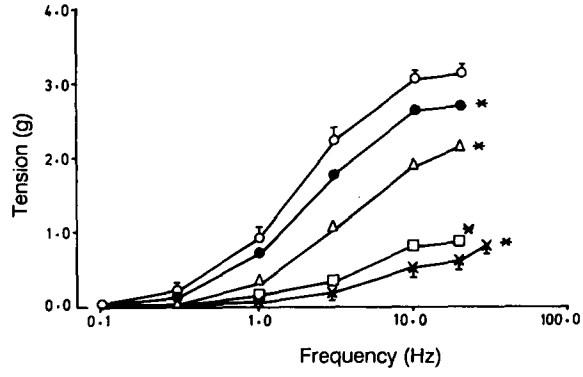

FIG. 1. Mean (±s.e.m. n=5 in each group) contractions of rat bladder strips to graded increases in the frequency of nerve stimulation. Responses in the absence (control, ○) or in the presence of antagonists: 1×10^{-7} g mL^{-1} atropine, 1×10^{-7} g mL^{-1} propranolol, 1×10^{-7} g mL^{-1} prazosin (●), and in presence of antagonists with dopamine in concentrations of 1×10^{-7} (△), 1×10^{-6} (□), or 1×10^{-5} g mL^{-1} (×). * $P<0·01$ compared with control by Student's unpaired *t*-test.

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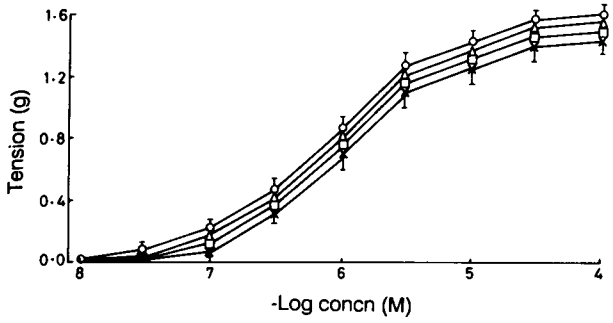

FIG. 2. Mean contractions of rat bladder strips (n = 5 in each group) to graded increases in concentration of ATP; vertical lines show s.e.m. Responses in the absence (control, ○) or in presence of 1×10^{-7} (△), 1×10^{-6} (□) and 1×10^{-5} g mL^{-1} (×) dopamine.

maximum response after the addition of 1×10^{-7} g mL^{-1} of either atropine, propranolol or prazosin (Fig. 1). The nerve-mediated contraction of the rat bladder resistant to these cholinergic and adrenergic antagonists was depressed ($P < 0.01$) by dopamine in a dose-related fashion. The depressions induced by dopamine consisted of rightward shifts in the log frequency-response curves that were associated with decreases in response to the highest frequencies used, the latter being comparable for 1×10^{-6} and 1×10^{-5} g mL^{-1} dopamine (Fig. 1).

ATP contracted the rat bladder in a dose-related fashion. The log dose-response curve of ATP was unaffected ($P > 0.05$) by dopamine administered in concentrations up to 1×10^{-5} g mL^{-1} (Fig. 2).

The concentration-dependent contraction of the rat bladder evoked by KCl was depressed to a similar extent by the three doses of dopamine administered. The depression consisted of rightward shifts ($P < 0.01$) of the log dose-response curves and decreases ($P < 0.05$) in the maximum response to KCl (Fig. 3).

Administration of dopamine alone in concentrations up to 1×10^{-3} g mL^{-1} did not produce any agonist effect on the bladder. However, the depressant action on NANC nerve stimulation of 1×10^{-6} g mL^{-1} dopamine was partly reversed by 1×10^{-6} g mL^{-1} haloperidol administered 5 min before dopamine (Fig. 4), indicating dopaminergic receptors partly mediate the depressant action of dopamine.

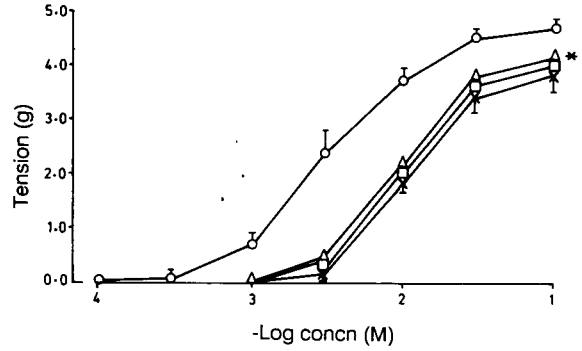

FIG. 3. Mean contractions of rat bladder strips (n = 5 in each group) to graded increases in concentration of KCl; vertical lines show s.e.m. Responses in the absence (control, ○) or in presence of 1×10^{-7} (△), 1×10^{-6} (□), and 1×10^{-5} g mL^{-1} (×) dopamine. * $P < 0.05$ compared with the control by Student's unpaired t-test.

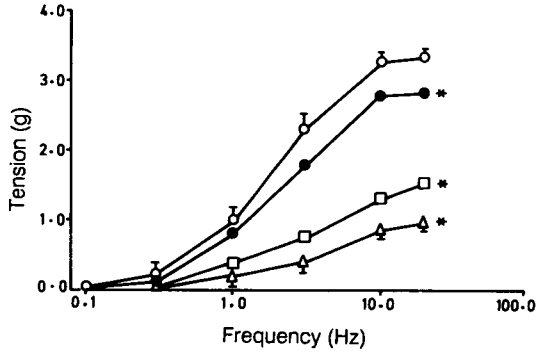

FIG. 4. Mean contractions of rat bladder strips (n = 5 in each group) to graded increases in the frequency of nerve stimulation; vertical lines show s.e.m. Responses in the absence (control, ○), or in the presence of 1×10^{-7} g mL^{-1} atropine, 1×10^{-7} g mL^{-1} propranolol, 1×10^{-7} g mL^{-1} prazosin (antagonists, ●); antagonists and 1×10^{-6} g mL^{-1} dopamine (△), antagonists, 1×10^{-6} g mL^{-1} dopamine and 1×10^{-6} g mL^{-1} haloperidol (□). * $P < 0.05$ compared with control by Student's unpaired t-test.

Discussion

The nerve-mediated contraction of the rat bladder was resistant to atropine (1×10^{-7} g mL^{-1}), propranolol (1×10^{-7} g mL^{-1}) and prazosin (1×10^{-7} g mL^{-1}), indicating the presence of NANC excitatory transmission in this tissue. The presence of NANC excitatory nerves has previously been demonstrated in various mammalian bladders (Dumsday 1971; Burnstock et al 1972; Brown et al 1979; Lot & Bennett 1982), and one putative transmitter for such nerves has been suggested to be ATP or a related purine compound. Results from the present study are therefore consistent with previous findings and also show that NANC nerves account mainly for excitatory nerve transmission in the bladder, making this a suitable preparation for studying the effects of dopamine on excitatory NANC transmission.

Dopamine was found to be potent in depressing NANC neurotransmission in the bladder; the effect depending on the dose of dopamine administered. Such observations have not been reported previously despite the different effects of dopamine on the autonomic nervous system reported in the literature (Goldberg 1972; Makabali et al 1982; Brown et al 1985; Mitchell et al 1987). Some of the possible mechanisms by which dopamine could depress NANC neurotransmission were examined in the present study. The possible role of purinoceptors was examined using the purinergic agonist, ATP. The existence of prejunctional P_1-purinoceptors and postjunctional P_2-purinoceptors has been described in many tissues and has been divided into several receptor subtypes (Bruns et al 1980; Burnstock 1980, 1991; Londos et al 1980; Burnstock & Buckley 1985; Blakeley et al 1988). The finding that dopamine did not affect contractions of the rat bladder evoked by ATP demonstrates that dopamine does not have a direct relaxant effect on smooth muscle and its depressant effect on NANC contractions is therefore not due to postsynaptic functional antagonism. Thus, depression of NANC neurotransmission by dopamine does not appear to be mediated by blockade of postjunctional purinergic receptor subtypes.

When dopamine was administered alone, it did not exert any demonstrable effect. A direct effect of dopamine on smooth muscle which is only evident when tone is raised seems unlikely as dopamine-induced depression of potassium-evoked contractions shows no dose dependency, whereas depression of NANC contractions by the same three concentrations of dopamine shows graded degrees of inhibition. Since the results with ATP

(Fig. 2) appear to rule out a postsynaptic inhibitory effect for dopamine, the most likely explanation for the dopamine inhibition of K^+-induced contractions is that these contractions are, at least in part, neurogenic and are therefore susceptible to presynaptic inhibition. This explanation is consistent with the mode of KCl-evoked contractions of smooth muscle. It has been suggested that about 30% of smooth muscle contraction evoked by KCl could be attributed to nerve stimulation (Bolton 1979). If dopamine depresses this component, it could account for depression of KCl-induced contractions as seen in the present study. This is possible since it has been shown that stimulation of prejunctional receptors in the bladder could cause a reduction in the release of excitatory transmitters (Maggi et al 1985). This possibility would require further investigation.

Results from the present study suggest that dopaminergic receptors might be involved in mediating the depressant action of dopamine on NANC neurotransmission since its depressant action was partially reversed by the dose of haloperidol used. Although haloperidol can antagonize both postsynaptic D_1- and presynaptic D_2-autoreceptor subtypes for dopamine (see Szmigielski et al 1984), results of the present study are consistent with a presynaptic site of action for dopamine. Taken together, these observations would suggest that dopamine appears to stimulate presynaptic D_2-receptors causing inhibition of NANC transmitter release.

The present study has shown that dopamine can depress NANC excitatory neurotransmission in the rat bladder. Although the possible mechanisms are still under investigation, the depressant action of dopamine is not mediated by purinoceptor blockade but appears to be mediated, at least in part, by stimulation of dopaminergic receptors.

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