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Mechanisms of Action of Dopamine in the Peripheral Nervous System of Chicks and Rats

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Abstract—The effects and mechanisms of action of dopamine on the lower oesophagus and expansor secundariorum muscle (ESM) of chicks and on the bladder of rat have been examined. Dopamine contracted the lower oesophagus in a concentration-related fashion but failed to contract the muscle from chicks pretreated with reserpine or *p*-chlorophenylalanine. Contraction of the ESM by dopamine was antagonized by prazosin but not by propranolol. Supersensitivity of the ESM to dopamine observed 3 or 28 days after surgical denervation of the muscle was comparable. Dopamine did not exert any agonist effect on the rat bladder but depressed responses to stimulation of non-adrenergic, non-cholinergic (NANC) nerves in the bladder. These findings indicate that dopamine contracts the upper oesophagus of chicks by releasing 5-hydroxytryptamine, activates α-adrenergic receptors causing contraction of the ESM but depresses NANC neurotransmission in the bladder.

Lot (1993b) showed that some of the actions of dopamine on the gastrointestinal tract of chicks differed from those of acetylcholine and noradrenaline. Moreover, dopamine has recently been shown to depress non-adrenergic, non-cholinergic (NANC) neurotransmission in the rat bladder (Lot 1993a). The aim of the present study was therefore to investigate the mechanisms by which dopamine produces its actions on peripheral, non-vascular smooth muscle.

Materials and Methods

Male chicks (Light Sussex and Rhode Island Red cross) obtained from the National Veterinary Research Institute, Vom, Nigeria, were used for studies on the lower oesophagus and the expansor secundariorum muscle (ESM). Adult male Wistar rats, 240–280 g, obtained from the National Veterinary Research Institute, Vom, Nigeria, were used for studies on the bladder.

The lower oesophagus of chicks

For studies involving the lower oesophagus, chicks aged 2–7 days after hatching were used. Chicks age- and weight-matched at the beginning of the experiment were divided into four experimental groups. One group received 5 mg kg^{-1} reserpine intraperitoneally, a second group received 10 mg kg^{-1} reserpine, while the third group received the equivalent volume of saline (control) before the chicks were all starved overnight. The fourth group received 100 mg kg^{-1} *p*-chlorophenylalanine and were starved overnight on the second day after administration of the drug. After starving overnight, chicks were killed with an overdose of ether. The thorax of each chick was cut open and the lower oesophagus quickly dissected out without stretching and placed in a beaker containing Krebs solution continuously bubbled with 95% O_2–5% CO_2. A 2-cm length of the lower oesophagus was threaded at both ends. One end was tied to a pin held in a Perspex block and suspended in a 20-mL organ bath maintained at 32 ± 1°C, while the other end was tied to an isotonic transducer connected to a flat-bed pen recorder (Servoscribe).

The ESM of chicks

The effect of surgical denervation on response of the ESM to agonist drugs was investigated. Chicks (30 days old) were anaesthetized with ether, the skin above the branch of the radial nerve supplying the ESM was incised and the nerve cut 1–2 cm from the muscle of the left wing with a pair of sterile scissors as described by Bennett et al (1982). The wound was sealed with an antiseptic spray dressing. The ESM from the right wing was left intact to serve as the control. The chicks were killed at the age of 33 or 58 days to study the effects of denervation.

For organ bath studies, chicks were killed by an overdose of ether and the ESM quickly dissected out. The ESM was freed of adherent tissue, except for the tendon and a piece of secondary feather at the base of the muscle. The feather was tied to a pin held in a Perspex block and suspended in a 20-mL organ bath maintained at 37 ± 1°C as described by Bennett et al (1982). The tendon was threaded 3 cm from the feather and tied to an isometric transducer connected to a Grass polygraph recorder (Model 7D).

Rat bladder

Rats were killed by stunning and bleeding, the bladder quickly dissected and cut open. A portion 3 mm wide and 7 mm long taken from the bladder body was threaded at both ends and set up in a 20-mL organ bath maintained at 37 ± 1°C 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).

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

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every 4 min. The frequency of stimulation was varied as desired. These parameters have been shown to stimulate the nerves of the bladder selectively (Lot & Bennett 1982).

Organ bath studies

The organ baths 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 the temperatures indicated above, by means of a water jacket connected to a thermostatically controlled pump (Haake). Tissues were given 30 min to equilibrate before experiments started.

Drug solutions were freshly prepared in concentrations such that the addition of 0·2 mL gave the required final bath concentration. Ascorbic acid (10^{-4} M) was added to dilute solutions of noradrenaline and dopamine. 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 were applied 5 min before agonist drugs, where they were used.

Drugs

Drugs used were acetylcholine bromide (BDH, Poole, UK), atropine sulphate (Sigma, Poole, UK), dopamine hydrochloride (Sigma), isoprenaline hydrochloride (BDH), DL-*p*-chlorophenylalanine (Sigma), phenylephrine hydrochloride (Sigma), prazosin hydrochloride (Sigma), propranolol hydrochloride (ICI, Macclesfield, Cheshire), reserpine (Serpasil, Ciba-Geigy, Summit, NJ).

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 by Student's unpaired *t*-test.

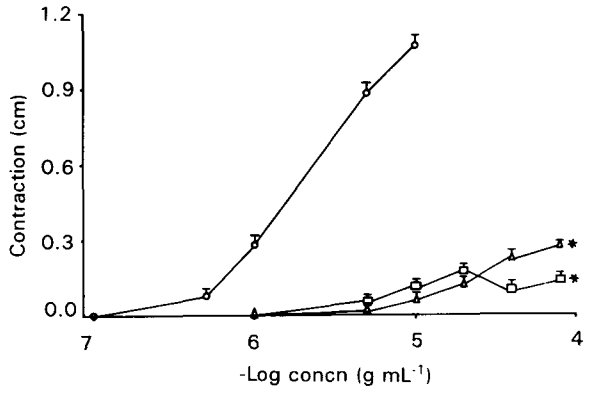

FIG. 1. Mean contractions of the lower oesophagus from chicks (n = 5 in each group) to graded increases in concentration of dopamine; vertical lines show s.e.m. Responses of the oesophagus of saline-treated chicks (control, ○) and chicks treated with 5 mg kg^{-1} reserpine (△) or 100 mg kg^{-1} *p*-chlorophenylalanine (□). *$P < 0.05$ compared with control.

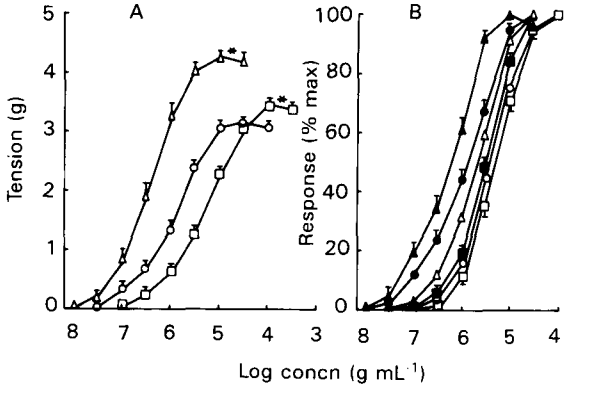

FIG. 2. Mean contractions of the expansor secundariorum muscle (ESM) (n = 6 in each group) from chicks aged 58 days to graded increases in concentration of dopamine, noradrenaline or isoprenaline; vertical lines show s.e.m. Responses to dopamine of normal ESMs (○) or ESMs from 58-day-old chicks denervated 28 days previously (●); normal ESM responses to noradrenaline (△) or responses of ESMs from 58-day-old chicks denervated 28 days previously (▲); and normal ESM responses to isoprenaline (□) or responses of ESMs from 58-day-old-chicks denervated 28 days previously (■). A. Response as tension (g). B. Response as % maximum tension. *$P < 0.05$ compared with dopamine.

Results

In saline-treated (control) chicks, dopamine produced a concentration-related contraction of the lower oesophagus which was abolished ($P < 0.05$) by pretreatment with 5 mg kg^{-1} reserpine or 100 mg kg^{-1} *p*-chlorophenylalanine (Fig. 1). The extent to which reserpine and *p*-chlorophenylalanine inhibited dopamine-induced contractions of the oesophagus were comparable (Fig. 1). However, when the concentration of reserpine was increased to 10 mg kg^{-1} there was no further increase ($P > 0.05$) in its inhibitory effect to the agonist action of dopamine.

The ESM was contracted by noradrenaline, dopamine and isoprenaline in a dose-related fashion. The order of sensitivity of the ESM to these agonists was noradrenaline > dopamine > isoprenaline, while the order for the maximum responses was noradrenaline > isoprenaline > dopamine (Fig. 2A); these differences were significant ($P < 0.05$). Surgical denervation for 28 days did not change ($P > 0.05$) the sensitivity of the ESM to isoprenaline (Fig. 2B). The denervated ESM was supersensitive ($P < 0.05$) to both noradrenaline and dopamine although the muscle was more responsive to noradrenaline (Fig. 2B). These data show some differences in response of the ESM to noradrenaline, dopamine and isoprenaline.

When ESMs of 30-day-old chicks were denervated for only 3 days, they became supersensitive ($P < 0.05$) to dopamine compared with the control (non-denervated) muscles (data not shown). This observation shows that supersensitivity of the ESM to dopamine could be attributed to the acute loss of its noradrenergic nerves.

Contractions of normal or denervated ESMs produced by noradrenaline and dopamine were antagonized by 10^{-7} g mL^{-1} prazosin, although this concentration had no effect on contractions by isoprenaline. Contractions of normal or denervated ESMs to isoprenaline were antagonized by 10^{-7} g

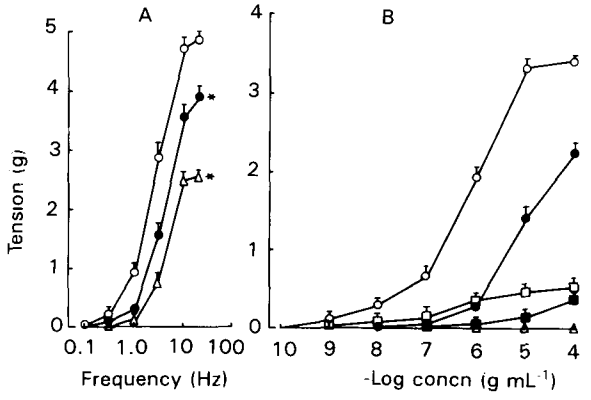

FIG. 3. A. Mean (±s.e.m., n=6 in each group) contractions of rat bladder strips to graded increases in the frequency of nerve stimulation. Responses in the absence (control, ○) or in the presence of antagonists: 10^{-7} g mL^{-1} atropine, 10^{-7} g mL^{-1} propranolol, 10^{-7} g mL^{-1} prazosin (●), and antagonists with 10^{-6} g mL^{-1} dopamine (△). B. Mean contractions of rat bladder strips (n=6 in each group) to graded increases in concentration of acetylcholine (○), acetylcholine in the presence of 10^{-7} g mL^{-1} atropine (●), phenylephrine (□), phenylephrine in the presence of 10^{-7} g mL^{-1} prazosin (■) and dopamine or isoprenaline (△). *$P<0.01$ compared with control.

mL^{-1} propranolol which had no effect on contractions produced by noradrenaline or dopamine.

Field stimulation caused a frequency-related contraction of the rat bladder (Fig. 3A). The contractions were reduced ($P<0.05$) by the addition of 10^{-7} g mL^{-1} each of atropine, prazosin and propranolol (Fig. 3A) used to block muscarinic, α-adrenergic and β-adrenergic receptors, respectively, in the bladder. The concentration-related contractions of the bladder in the presence of these antagonists has been attributed to stimulation of its NANC nerves (Burnstock et al 1972; Lot & Bennett 1982). In the presence of the receptor antagonists, 10^{-6} g mL^{-1} dopamine reduced ($P<0.05$) responses to NANC nerve stimulation (Fig. 3A).

Administration of acetylcholine produced a concentration-related contraction of the bladder that was antagonized ($P<0.05$) by 10^{-7} g mL^{-1} atropine (Fig. 3B). Phenylephrine caused only weak contractions of the bladder compared with those produced by acetylcholine and the phenylephrine-induced contractions were antagonized ($P<0.05$) by 10^{-7} g mL^{-1} prazosin (Fig. 3B). In concentrations up to 10^{-4} g mL^{-1}, both dopamine and isoprenaline failed to contract ($P<0.05$) the bladder (Fig. 3B).

Discussion

Lot (1993b) showed that lysergic acid diethylamide (LSD) abolished contraction of the lower oesophagus of chicks induced by dopamine, and that repeated administration of a large dose of dopamine caused tachyphylaxis. The finding indicated that dopamine probably contracts the lower oesophagus by releasing 5-hydroxytryptamine (5-HT). This possibility has been further examined in the present study using reserpine and *p*-chlorophenylalanine.

Reserpine causes depletion of monoamines (noradrenaline, dopamine and 5-HT) at various sites in the body (Rang & Dale 1991). Depletion of monoamines by reserpine could therefore lead to failure of transmission, especially with compounds which act by releasing monoamines. Results from the present study are consistent with this since they show that administration of reserpine inhibited the ability of dopamine to contract the lower oesophagus, suggesting that dopamine acts in this tissue by releasing monoamines. In this regard, administration of 5 and 10 mg kg^{-1} reserpine both inhibited dopamine-induced contractions of the oesophagus to similar extents, indicating that 5 mg kg^{-1} reserpine produced the maximum inhibitory effect. Although noradrenaline, dopamine and 5-HT all contracted the lower oesophagus, it appears unlikely that noradrenaline mediates the effect of dopamine since prazosin did not antagonize the effect of dopamine on the oesophagus (Lot 1993b).

p-Chlorophenylalanine selectively and irreversibly inhibits tryptophan hydroxylase, thereby inhibiting the synthesis of, and causing depletion of 5-HT (Rang & Dale 1991). The finding in this study that *p*-chlorophenylalanine was as effective as reserpine in inhibiting dopamine-induced contraction of the lower oesophagus suggests that the inhibition caused by reserpine can also be attributed to depletion of 5-HT in this tissue. Thus, depletion of 5-HT by reserpine and *p*-chlorophenylalanine appears to be responsible for abolishing dopamine-induced contraction of the lower oesophagus. These findings, together with the observation that LSD inhibited contractions of the lower oesophagus induced by dopamine and 5-HT (Lot 1993b), therefore confirm that dopamine contracts the lower oesophagus by releasing 5-HT.

In spite of this proposed mechanism of action for dopamine, it is pertinent to mention that dopamine might not act only through 5-HT release in the lower oesophagus. This suggestion is based on the observation that moderate doses of haloperidol or pimozide antagonized dopamine-induced contractions of the lower oesophagus (Lot 1993b), suggesting that dopaminergic receptors might also be involved in mediating that action of dopamine.

The observation that dopamine and noradrenaline both relaxed the duodenum, ileum, large intestine and caecum of chicks (Lot 1993b) suggests that the two amines might produce some of their actions by a similar mechanism. This suggestion is further strengthened by reports that dopamine produces some of its effects by stimulating cardiac β-adrenoceptors, and by α-adrenoceptor stimulation achieved by direct activation or indirectly through noradrenaline release (Farmer 1966; Goldberg 1972). The possibility of dopamine acting in a similar fashion to noradrenaline has been examined in the present study using the isolated ESM, a smooth muscle in the wing of chicks wholly innervated by noradrenergic nerves (Buckley & Wheater 1968; Bennett & Malmfors 1970; Lot & Bennett 1982). Noradrenaline, dopamine and isoprenaline were found to contract the ESM. The contraction produced by noradrenaline and dopamine was antagonized by prazosin, a known α-adrenergic receptor antagonist, indicating they were mediated by activation of α-adrenergic receptors. The presence of β-adrenergic receptors causing contraction of the ESM has previously been demonstrated (Lot & Udoh 1991) and has been confirmed in the present study. The inability of propranolol to antagonize dopamine-induced contraction of the ESM indicates that dopamine does not activate β-adrenergic receptors in the ESM.

Surgical denervation of the ESM causes loss of its noradrenergic nerves (Bennett et al 1982; Lot & Udoh 1991).

This intervention could therefore be used to assess whether dopamine-induced contraction of the ESM is due to direct activation of α-adrenoceptors. Denervation has been reported to induce supersensitivity of the ESM to noradrenaline (Bennett et al 1982; Lot & Udoh 1991) but not to isoprenaline (Lot & Udoh 1991) and these observations were confirmed in the present study. It was also found in this study that denervation for 28 days, which causes a long-lasting loss of noradrenergic nerve terminals, induced supersensitivity of the ESM to dopamine indicating that dopamine directly activates α-adrenergic receptors. In this regard, the actions of dopamine would resemble those of noradrenaline. To determine whether postjunctional changes (Bennett et al 1982) might contribute to denervation supersensitivity of the ESM to dopamine, the muscles from some chicks were denervated for only 3 days, since it has been reported that surgical denervation of the ESM for only 3 days causes loss of its noradrenergic nerves without accompanying postjunctional changes (Campbell et al 1977). Using this model, it was found that the ESM was as supersensitive to dopamine 3 days after surgery as the supersensitivity seen 28 days after surgery. This finding confirms that dopamine causes contraction of the ESM in the absence of its noradrenergic nerves; therefore, it is a direct-acting α-adrenoceptor agonist in the ESM. The findings also indicate that supersensitivity to dopamine can be attributed to the acute loss of noradrenergic nerves, rather than to denervation-induced postjunctional changes in the ESM.

Recently, a third component of the autonomic nervous system that is non-adrenergic, non-cholinergic (NANC) has been identified. The possible effects of dopamine on NANC transmission have 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). The nerve-mediated contraction of the rat bladder was resistant to atropine (10^{-7} g mL^{-1}), propranolol (10^{-7} g mL^{-1}) and prazosin (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). Results from the present study are therefore consistent with such 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.

In the presence of antagonists used to block cholinergic and adrenergic receptors, dopamine blocked responses to NANC nerve stimulation. However, dopamine did not exert any direct agonist action when administered in concentrations up to 10^{-4} g mL^{-1}. In this regard, the action of dopamine on the rat bladder was similar to that of isoprenaline, but differed from those of acetylcholine and phenylephrine which both contracted the bladder by stimulating muscarinic and α-adrenergic receptors, respectively. The finding that acetylcholine produced more pronounced contractions of the bladder than phenylephrine is consistent with the known pharmacology of this preparation, being predominantly under parasympathetic control (Rang & Dale 1991). However, the mechanism by which dopamine depresses NANC transmission in the rat bladder, as observed in this study, has been further examined and appears to be partly mediated by dopaminergic receptors (Lot 1993a).

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