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The effects of nicotine on spontaneous contractions of cat urinary bladder *in situ*

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1 Nicotine and dimethyl-phenylpiperazinium (DMPP) increased intravesicular pressure and then transiently depressed the spontaneous activity of the urinary bladder in chloralose anaesthetized cats.

2 Adrenaline (5–10 $\mu g\,kg^{-1}$), noradrenaline (5–20 $\mu g\,kg^{-1}$) and isoprenaline (40–50 $\mu g\,kg^{-1}$) which depressed spontaneous urinary bladder activity, were antagonized by the β-receptor blocking agent propranolol (1 $mg\,kg^{-1}$). Phenylephrine (10–30 $\mu g\,kg^{-1}$) was ineffective on the urinary bladder though it increased the systemic blood pressure. This latter effect was blocked by the α-receptor blocking agent phentolamine (2 $mg\,kg^{-1}$).

3 Acetylcholine (2–8 $\mu g\,kg^{-1}$) caused a marked fall in systemic blood pressure, which was potentiated by physostigmine, but failed to produce any response on the intravesicular pressure even after physostigmine (50–100 $\mu g\,kg^{-1}$) treatment.

4 ATP (2 $mg\,kg^{-1}$) produced an increase in intravesicular pressure accompanied by a fall in systemic blood pressure. The increased intravesicular pressure was antagonized by quinidine (20 $mg\,kg^{-1}$); however, the fall in blood pressure remained unaltered.

5 The increased intravesicular pressure induced by nicotine (20–40 $\mu g\,kg^{-1}$) or DMPP (50–100 $\mu g\,kg^{-1}$) was not affected by phentolamine (2 $mg\,kg^{-1}$), propranolol (1 $mg\,kg^{-1}$) or guanethidine (15–20 $mg\,kg^{-1}$). Physostigmine (50–100 $\mu g\,kg^{-1}$), hemicholinium 3 (2 $mg\,kg^{-1}$) or atropine (1 $mg\,kg^{-1}$) were also unable to affect the response to nicotine.

6 Hexamethonium (1 $mg\,kg^{-1}$), reduced the amplitude of spontaneous bladder contractions and quinidine (20 $mg\,kg^{-1}$) abolished the effect of nicotine.

7 Bilateral sectioning of the cervical sympathetic or hypogastric nerves did not alter the effect of nicotine or DMPP. Higher spinal cord transection (C_1–C_2) blocked the spontaneous, as well as the nicotine- and DMPP-induced, contractions of the bladder.

8 It is concluded that the increase in intravesicular pressure induced by nicotine is atropine-resistant and is not mediated either through adrenergic or cholinergic mechanisms. It is probable that a purinergic mechanism is involved, via the activation of P_2-receptors present in the urinary bladder.

Introduction

The mammalian urinary bladder is innervated by both hypogastric and pelvic nerves (Langley & Anderson, 1895; 1896). Although the bladder possesses some spontaneous and myogenic activity, nervous control is important for its normal function in the micturition reflex (Callahan & Creed, 1981).

The excitatory responses of the mammalian urinary bladder to stimulation of sacral parasympathetic nerves (Langley & Anderson, 1895; Henderson & Roepke, 1934; 1935; Ambache, 1955; Ursillo, 1961; Goldenberg, 1965; Ambache & Zar, 1970) and the muscarinic receptors themselves are atropine-resistant (Dale & Gaddum, 1930; Elmer, 1975; Carpenter, 1977). Although the smooth muscle of the bladder contains few adrenergic nerves (Gosling & Dixon, 1975), hypogastric nerve stimulation can produce a powerful contraction of the bladder (Edvardsen & Setekleiv, 1968). These observations suggest that either other nerve types are involved or the actions of nerve stimulation are indirect (Creed, 1979). The presence of non-adrenergic non-cholinergic (NANC) excitatory fibres in the pelvic

and hypogastric outflows has been suggested (Henderson & Roepke, 1934; Chesher & James, 1966; Ambache & Zar, 1970; Dumsday, 1971; Burnstock *et al.*, 1972). These NANC fibres may be purinergic (Burnstock *et al.*, 1972; 1978; Dean & Downie, 1978; Burnstock, 1980).

Nicotine and dimethyl-phenylpiperazinium (DMPP) have been shown to contract and then relax the cat urinary bladder *in situ* (Edmunds & Roth, 1920; Goldenberg, 1965). In dogs and cats an indirect excitation of autonomic ganglia may be responsible for the vesicular motility induced by nicotine and DMPP (Chen *et al.*, 1954; Gyermek, 1961; Larson *et al.*, 1961; Garret, 1963; Goldenberg, 1965; Sjöstrand *et al.*, 1972), parasympathetic ganglia in the pelvic plexus that discharge motor impulses to the bladder; sympathetic ganglia are probably not involved (Vanov, 1965). However, Goldenberg (1965) concluded that the contractions of the urinary bladder to nicotine are due to stimulation of parasympathetic nerves but the transmitter liberated from these nerves must be a substance other than acetylcholine, histamine or 5-hydroxytryptamine.

There are various opposing opinions regarding the cholinergic and adrenergic mechanisms of vesicular motility in normal physiological processes as well as those produced by drugs (Goldenberg, 1965; Vanov, 1965). Since the nicotine-induced increase in vesicular motility occurs in both atropine-treated animals and in intact animals in which the hypogastric nerves have been sectioned (Edmunds & Roth, 1920; Vanov, 1965; Goldenberg, 1965), the present study investigated the sensitivity of the bladder to nicotine and the role of autonomic nerves in its motility in the cat.

Methods

Experiments were performed on 42 adult cats (2 to 2.5 kg), anaesthetized with chloralose (60–70 mg kg^{-1} i.v.) after induction with ether. A 'T'-shaped polyethylene cannula was inserted in the trachea after a low tracheotomy and connected to a thermistor probe to monitor the respiratory rate. Variations in the temperature of the inspired and expired air, recorded on a Beckman RM Dynograph via the thermistor, indicated respiratory rate. The femoral artery was cannulated for continuous recording of systemic blood pressure which was measured with a Bell and Howell (Type 4–327–0129) pressure transducer. The nictitating membrane was connected with silk thread to a Beckman isometric force displacement transducer (Type 4151) and the contraction was recorded after prior amplification through the Beckman V/P/P coupler (Type 9853 A) on a Beckman RM Dynograph.

Denervation and spinal cord transection

The cervical sympathetic nerves were isolated with the aid of a stereoscopic dissecting microscope (Vicker's) and bilateral denervation performed below the nodose ganglion after placing a small ice cube over the nerves to prevent cardiac shock. The hypogastric nerves were exposed retroperitoneally as described by Floyd *et al.* (1977) and sectioned below the inferior mesenteric ganglion. Transection of the spinal cord at the level of C_1-C_2 vertebrae was performed in 6 cats following the methods of Koley & Mukherjee (1964). The spinal cord was opened by laminectomy and before transection a 2% solution of lignocaine was injected in the cord at the C_1-C_2 level to avoid spinal shock. Animals were artificially respired when required, and normal physiological saline (0.9% w/v NaCl solution) with 5% glucose was administered by drip feed into the femoral vein to maintain body fluid and stabilize the preparation.

Adrenalectomy

Adrenalectomy was performed in 5 cats as described by Armitage (1965). The adrenal veins and arteries were isolated carefully with the aid of a stereoscopic dissecting microscope. Double ties were placed around the adrenal veins and arteries and the glands removed. The animals were allowed to rest for about 60 min after the surgical procedure.

Intravesicular pressure recording

The urethra was exposed by a midline incision of the lower abdomen and cleared of surrounding tissues. A polyethylene catheter was inserted into the urinary bladder via the urethra to record intravesicular pressure. The bladder was first emptied and then filled with 10 to 20 ml of physiological saline introduced through the catheter. The intravesicular pressure changes due to spontaneous and drug-induced bladder contractions were measured by a Bell and Howell (Type 4–327–0129) pressure transducer and recorded on a Beckman RM Dynograph.

Drugs used

Drugs, dissolved in physiological saline, were injected intravenously through a catheter in the femoral vein; doses refer to the salt. Drugs used were: acetylcholine hydrochloride (Sigma); physostigmine salicylate (Boehringer Ingelheim); nicotine hydrogen tartrate (BDH); propranolol hydrochloride (ICI); isoprenaline sulphate (Burroughs Welcome) phenylephrine hydrochloride (Sigma); guanethidine sulphate (Ciba-Geigy); hexamethonium bromide (Koch-Light Lab.); hemicholinium 3 (Aldrich); ad-

renaline acid tartrate (Burroughs Welcome); noradrenaline (Sigma); atropine sulphate (Bengal Immunity); lignocaine hydrochloride (Gesicain 2%, Suhrid-Geigy); dimethyl-phenylpiperazinium (DMPP, Fluka); phentolamine mesylate (Rogitine, Ciba-Geigy); quinidine sulphate (Burroughs Welcome); adenosine 5'triphosphate (ATP, Sigma).

Statistical analysis of data

Results are expressed as mean ± standard error of mean (s.e.mean). The significance of differences between groups was estimated using Student's *t* test.

Results

(1) *Responses to nicotine*

In anaesthetized animals, intravesicular pressure fluctuated with spontaneous bladder contractions. Nicotine (20–40 $\mu g\,kg^{-1}$) increased bladder contractions, as evidenced from the sharp rise in intravesicular pressure. This contraction was maintained for about 30 to 40 s and was followed by a depression of both the frequency and amplitude of normal spontaneous bladder contractions. A typical pattern of the response to nicotine is shown in Figure 1. As illustrated, the increased bladder contractions correspond to the increased blood pressure and contraction of the nictitating membrane. The responses to nicotine were reproducible throughout the experimental period provided that the injections of nicotine were spaced 25 to 30 min apart. These observations indicate that bladder contractions are associated with a generalized postganglionic sympathetic excitation by nicotine as evident from the simultaneous increase in blood pressure and contraction of the nictitating membrane (Koley *et al.*, 1982). DMPP, a ganglionic stimulating agent produced similar responses to those of nicotine.

(2) *Responses to other drugs*

Adrenaline and noradrenaline Intravenous administration of adrenaline (5 to 10 $\mu g\,kg^{-1}$) and noradrenaline (5 to 20 $\mu g\,kg^{-1}$) increased systemic blood pressure but decreased or abolished the spontaneous contractions of the urinary bladder (Figure 2). Propranolol (1 $mg\,kg^{-1}$), a β-adrenoceptor blocker, abolished the depressive responses of the bladder to these catecholamines, indicating that the β-adrenoceptors play an inhibitory role in bladder contraction.

Phenylephrine and isoprenaline Phenylephrine and isoprenaline respectively caused a rise and fall of blood pressure. Phenylephrine (10–30 $\mu g\,kg^{-1}$), was ineffective on, while isoprenaline (40–50 $\mu g\,kg^{-1}$) greatly reduced, the spontaneous contractions of the

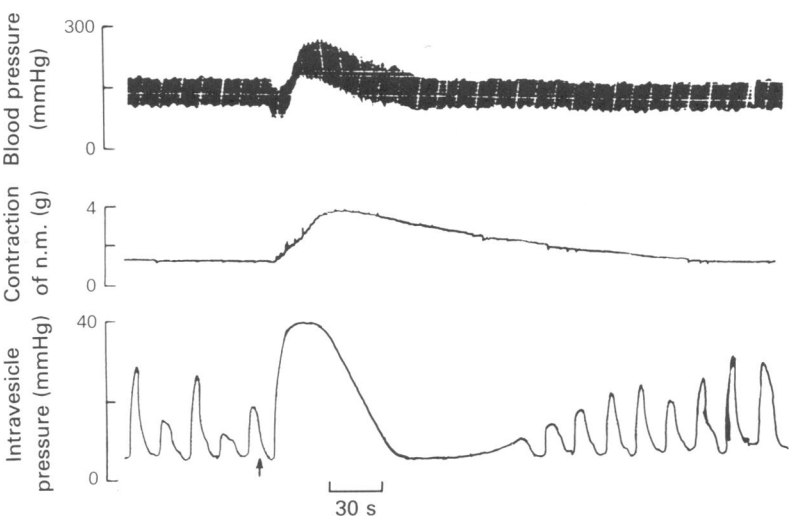

Figure 1 The effects of nicotine (40 $\mu g\,kg^{-1}$) on systemic blood pressure (top trace), nictitating membrane (n.m.) (middle trace) and intravesicle pressure (lower trace) in the anaesthetized cat. The rise in intravesicle pressure starts with the rise of blood pressure and the contraction of the nictitating membrane. The arrow indicates nicotine administration.

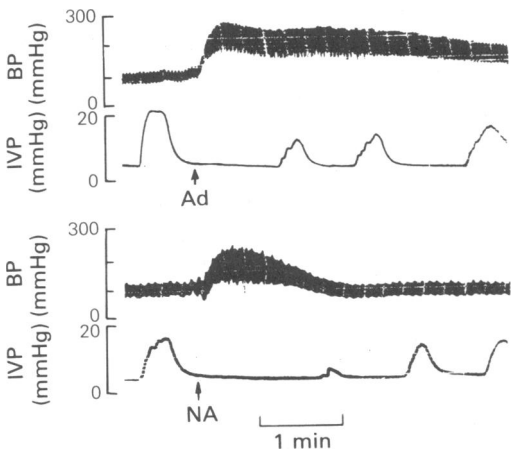

Figure 2 The effects on systemic blood pressure (BP—upper trace) and bladder intravesicle pressure (IVP—lower trace) of adrenaline, 10 $\mu g\,kg^{-1}$ (Ad, upper panels) and noradrenaline, 15 $\mu g\,kg^{-1}$ (NA, lower panels). Both these drugs depressed the spontaneous activity of the cat bladder.

bladder. The inhibitory effect of isoprenaline was abolished by propranolol (1 $mg\,kg^{-1}$) (Figure 3). These observations indicate that depression of bladder motility is mediated via β-adrenoceptor activation and indicate that the release of postganglionic sympathetic neurotransmitters or of catecholamines from the adrenal medulla cannot account for the increased bladder motility induced by nicotine.

Acetylcholine Acetylcholine (2–8 $\mu g\,kg^{-1}$) reduced systemic blood pressure significantly but had no effect on the spontaneous contractions of the urinary bladder. In the presence of physostigmine (50 $\mu g\,kg^{-1}$), an acetylcholinesterase inhibitor, the vasodepressor response to acetylcholine was potentiated but the spontaneous bladder contractions remained unaffected. Atropine (1 $mg\,kg^{-1}$), counteracted the acetylcholine-induced vasodepression, but was without effect on the spontaneous contractions.

Adenosine triphosphate ATP (2 $mg\,kg^{-1}$) increased intravesicular pressure immediately (5 to 7 s) after administration and reduced systemic blood pressure. Pretreatment of the animals with quinidine (20 $mg\,kg^{-1}$) abolished the effect of ATP on the urinary bladder but did not alter the blood pressure response (Figure 4). The urinary bladder became unresponsive to ATP (2 $mg\,kg^{-1}$) after 4 or 5 injections at intervals of 5 min.

(3) *Effects of different antagonists*

Phentolamine, propranolol and guanethidine Phentolamine (2 $mg\,kg^{-1}$), an α-receptor antagonist and propranolol (1 $mg\,kg^{-1}$), a β-receptor antagonist, had no effect on spontaneous bladder activity (although propranolol initially decreased blood pressure) and failed to antagonize the responses to nicotine in the urinary bladder. Guanethidine (20 $mg\,kg^{-1}$), an adrenergic neurone blocking agent, was also ineffective in this respect (Table 1). This

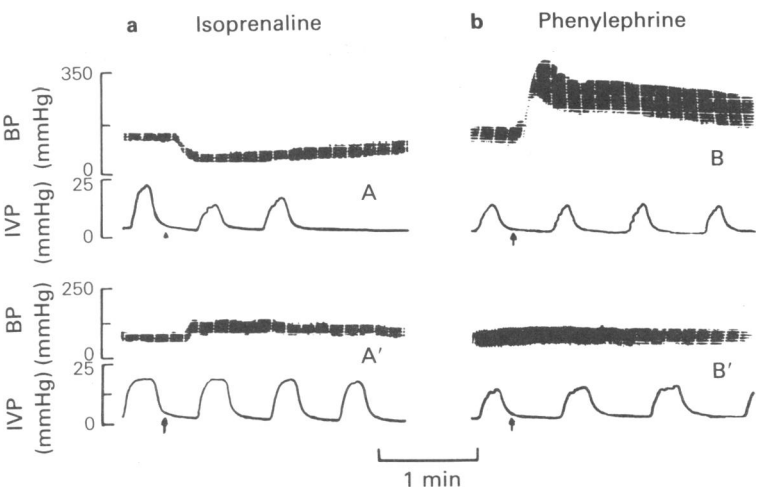

Figure 3 Changes in blood pressure (BP) and intravesicle bladder pressure (IVP) produced by (a) isoprenaline (50 $\mu g\,kg^{-1}$) and (b) phenylephrine (20 $\mu g\,kg^{-1}$) before (A,B) and after (A') propranolol (1 $mg\,kg^{-1}$) or (B') phentolamine (2 $mg\,kg^{-1}$) administration.

Table 1 Effect of guanethidine, phentolamine and propranolol on the increase in pressure in the cat urinary bladder induced by nicotine

Pretreatment	*Dose* ($mg\,kg^{-1}$)	*Responses of urinary bladder to nicotine (40 $\mu g\,kg^{-1}$)* (mm Hg)
None	—	40.25 ± 2.80 (12)
Guanethidine	20	42.40 ± 2.76 (5)*
Phentolamine	2	38.20 ± 2.55 (5)*
Propranolol	1	39.80 ± 2.43 (5)*

The values shown are mean ± s.e.mean with number of observations in parentheses. *$P < 0.1$, compared to control response to nicotine (no pretreatment).

suggests that the nicotine-induced bladder contraction is not a sympathomimetic effect.

Hemicholinium 3 and physostigmine Hemicholinium 3 ($2\,mg\,kg^{-1}$), which interferes with the synthesis of acetylcholine and hence the content in ganglia, failed to affect either the spontaneous activity of the bladder or the contractions induced by nicotine. The pressure responses to nicotine and to DMPP were also unaffected by physostigmine ($50-100\,\mu g\,kg^{-1}$) (Table 2).

Atropine, hexamethonium and quinidine Atropine ($1\,mg\,kg^{-1}$) was ineffective either on spontaneous bladder contractions or on those induced by nicotine and DMPP (Table 2). Hexamethonium ($1\,mg\,kg^{-1}$) reduced the amplitude of spontaneous bladder contractions. Nicotine and DMPP each failed to produce any response in animals pretreated with hex-

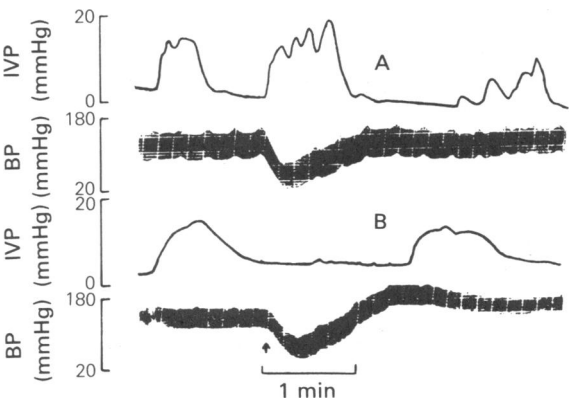

Figure 4 Changes in intravesicle pressure and blood pressure in response to ATP $2\,mg\,kg^{-1}$ before (A) and after (B) quinidine ($20\,mg\,kg^{-1}$). Note the absence of contraction of the cat bladder in trace B, although the ATP-induced hypotension remains unaltered.

Table 2 Effect of physostigmine, atropine, hemicholinium 3 and hexamethonium on the increase in pressure in the cat urinary bladder produced by nicotine and DMPP

Pretreatment	*Dose* (kg^{-1} body wt)	*Responses of urinary bladder* (mmHg) *Nicotine (40 $\mu g\,kg^{-1}$)*	DMPP ($50\,\mu g\,kg^{-1}$)
None		37.35 ± 1.49 (14)	44.66 ± 2.06 (15)
Physostigmine	50 μg	35.50 ± 1.16 (5)	40.20 ± 2.24 (5)
Atropine	1 mg	37.00 ± 3.36 (6)	41.16 ± 1.66 (6)
Hemicholinium 3	2 mg	35.00 ± 3.73 (6)	42.5 ± 4.20 (6)
Hexamethonium	1 mg	0 ± 0 (5)*	0 ± 0 (5)*

The results show mean ± s.e.mean of number of observations given in parentheses. *$P < 0.001$, compared to control responses (no pretreatment).

amethonium (Figure 5, Table 2). Quinidine had no effect on the spontaneous activity of the bladder but abolished the response to nicotine (Figure 6). Nicotine was also ineffective in producing any response on the ATP-desensitized urinary bladder.

(4) *Effects of adrenalectomy, denervation and spinal cord transection*

In animals in which both the adrenal glands were removed surgically, nicotine produced a rise in intravesicular pressure. Bilateral sectioning of the cervical sympathetic or of the hypogastric nerves did not alter either the spontaneous, nicotine- or DMPP-induced bladder contractions. In animals in which the spinal cord had been sectioned at the level of the C_1-C_2 vertebrae, the spontaneous bladder contractions were absent. Also, nicotine and DMPP both failed to contract the urinary bladder in such preparations (Figure 7), although their effects on blood pressure remained unaltered compared to those in untreated animals.

Discussion

The responses to nicotine, DMPP and biogenic amines of the urinary bladder of mammals have been extensively studied (Langley & Anderson, 1895; Elliot 1907; Edmunds & Roth 1920; Henderson & Roepke 1935; Edge 1955; Gyermek 1961; Vanov 1965, Elmer 1974; Creed 1979). Different sub-types of adrenoceptors on the wall of the urinary bladder exist but excitatory α-adrenoceptors (Edvardsen, 1968a; Tiara, 1972; Elmer, 1974; Creed, 1979) are sparse; phenylephrine failed to produce any contraction even in the presence of propranolol. Adrenaline, noradrenaline and isoprenaline were always inhibitory and each produced a propranolol-sensitive depression of spontaneous contractions confirming the presence of inhibitory β-adrenoceptors (Edvardsen 1968b; Edvardsen & Setekleiv 1968; de Groat, 1975, Hindmarsh *et al.*, 1977; Creed 1979).

The sudden increase followed by a decrease in intravesicular pressure produced by nicotine and DMPP in dogs, cats, rabbits and rats have been ascribed to an indirect action via autonomic ganglia from which the postganglionic motor nerves to the bladder arise (Chen *et al.*, 1954; Gyermek 1961; 1962; Garret 1963; Vanov 1965; Goldenberg 1965; Sjostrand *et al.*, 1972). In the cat urinary bladder *in situ*, stimulation of the hypogastric nerve caused a contraction followed by cessation of rhythmic contractions and by a reduction of bladder tone (relaxation) (Edvardsen 1968b; Edvardsen & Setekleiv 1968). The initial contraction is reduced by α-adrenoceptor antagonists and the subsequent relaxation is converted to a contraction by β-adrenoceptor antagonists (Edvardsen 1968b). In the present study α- and β-adrenoceptor antagonists failed to affect the responses to nicotine and DMPP as did transection of the cervical sympathetic nerves, the hypogastric nerves peripheral to the inferior mesenteric ganglion, or pretreatment with guanethidine (blocks the release of the adrenergic transmitter; Boura & Green, 1965). These results do not support the involvement of adrenergic sympathetic nerves in the contractile response. Since adrenergic fibres end on ganglion cells in the bladder wall (Hamberger & Norberg 1965; Gosling & Dixon, 1975), a direct action of nicotine and DMPP on the bladder muscle has already been considered but discounted by Hukovic *et al.* (1964). Failure of guanethidine to modify their effects on the bladder (in the present study) suggests that it is unlikely that the effects of these drugs are mediated via the stimulation of adrenergic fibres on ganglion cells. The inability of adrenalectomy to modify the response to nicotine implies that it is not

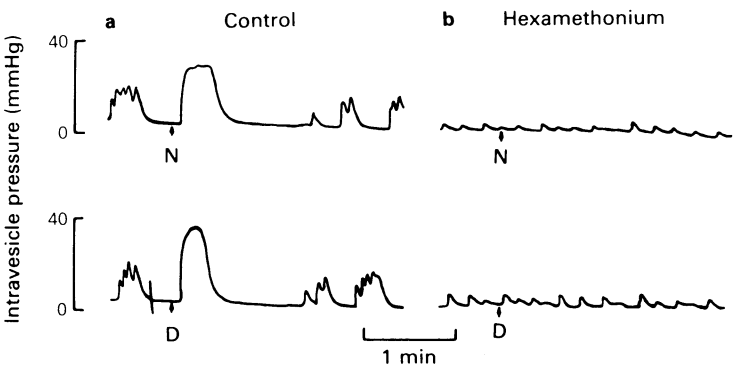

Figure 5 (b) The effect of pretreatment with hexamethonium (1 mg kg^{-1}) on intravesicle pressure responses produced by nicotine, N,40 μg kg^{-1} and DMPP (D, 50 μg kg^{-1}) compared to the control responses.

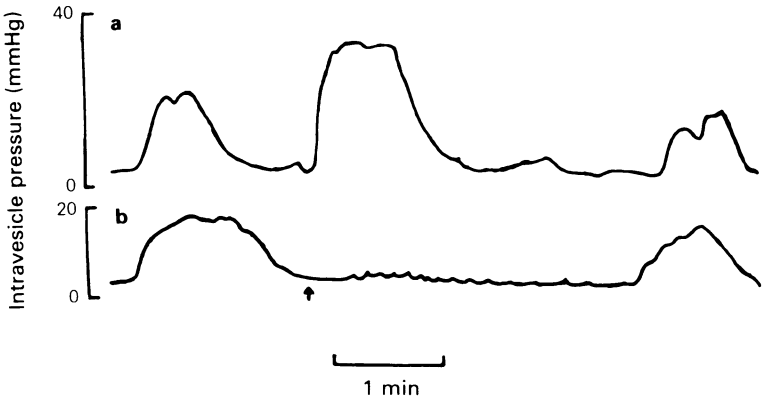

Figure 6 Effect of nicotine (↑) on the cat urinary bladder in the absence (a) and presence (b) of quinidine. Pretreatment with quinidine (b) temporarily abolished the nicotine-induced bladder contraction.

mediated through the release of catecholamines from the adrenal glands.

The responses to nicotine and DMPP were resistant to atropine (see also Goldenberg, 1965; Saum & de Groat, 1973) and unaffected by physostigmine and hemicholinium 3, indicating that acetylcholine was not involved in the effects of these drugs. Also, the ineffectiveness of atropine and physostigmine on the spontaneous contractions suggests that the mechanism responsible for these does not involve acetylcholine. However, hexamethonium reduced both the rhythmic spontaneous contractions and those to nicotine and DMPP. Nicotine may have to enter the nerve terminals in order to exert its effect (Burn & Gibbons, 1964; Su & Bevan, 1970; Westfall & Brasted, 1972) and blockade of the effect of nicotine by hexamethonium may arise from a block of its entry into the noradrenergic nerve terminals.

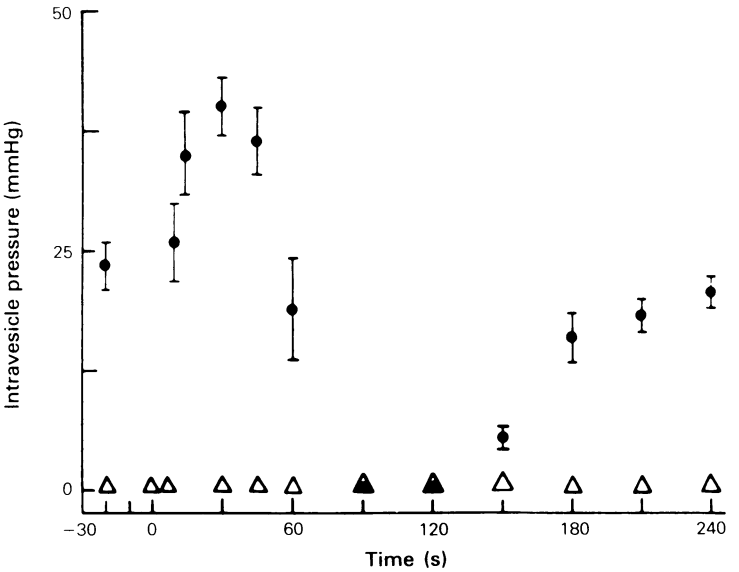

Figure 7 The changes in intravesicle pressure with time (s) produced by nicotine 40 $\mu g\,kg^{-1}$ (given at time 0) in control (●————●) and in acute spinal animals (△————△). Results show the mean and vertical lines s.e.mean of 6 preparations.

Quinidine (a P_2-receptor antagonist; Burnstock, 1980) also antagonized the nicotine response, an effect which could be due to the block of entry of nicotine into the noradrenergic or purinergic nerve terminals (see Theobald, 1983b).

The principal active substance released by non-adrenergic non-cholinergic nerves is a purine nucleotide, probably ATP and the nerves have therefore been termed purinergic (Burnstock, 1972; 1975; 1980; Burnstock *et al.*, 1978; Theobald, 1982; 1983a). The predominant response to ATP in smooth muscle is a relaxation; excitatory responses occur in the urinary bladder (Ambache & Zar, 1970; Burnstock *et al.*, 1972; Creed & Tulloch, 1982). P_2-purinoceptors predominate in the bladder (Burnstock, 1978; 1980) and these are antagonized by quinidine (Burnstock, 1980). This is supported in the present experiments; exogenous ATP increased bladder pressure (mimicking the response to nicotine) an effect antagonized by quinidine, a P_2-receptor antagonist (Burnstock, 1980). Quinidine also antagonized the effect of nicotine which suggests that nicotine releases ATP, from NANC nerves which then acts on the P_2-receptors (Burnstock 1978; 1980), to increase intravesicular pressure. According to Ganong (1977), in acute spinal preparations, the bladder becomes flaccid and unresponsive. This may be the possible cause for the absence of both spontaneous and nicotine- or DMPP-induced bladder contractions in transected animals in the present study.

In conclusion, the present data suggest that the cat urinary bladder receives a sparse adrenergic innervation comprising inhibitory β- but no excitatory α-adrenoceptors. The large spontaneous bladder contractions followed by inhibition of these contractions, induced by nicotine are unlikely to be mediated through α- or β-adrenoceptors or through cholinergic receptors, but purinergic (P_2)-receptors may be involved. The purinergic nerves may cause the release of ATP after administration of nicotine. The abolition of an autonomic neuroeffector response after spinal cord transection indicates that the response is not of supraspinal origin.

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