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Mechanism of action of nicotine in isolated urinary bladder of guinea-pig

Tetsuhiro Hisayama, Michiko Shinkai, [1]Issei Takayanagi & Toshie Toyoda

Department of Chemical Pharmacology, Toho University School of Pharmaceutical Sciences, 2-2-1, Miyama, Funabashi, Chiba 274, Japan

1 Nicotine produced a transient contraction of isolated strips of guinea-pig urinary bladder. The response to nicotine was antagonized by the nicotinic receptor antagonist, hexamethonium but was insensitive to tetrodotoxin.

2 The nicotine-induced contraction was potentiated by the cholinesterase inhibitor, physostigmine, and was reduced to 50% and 70% by the muscarinic cholinoceptor antagonist, atropine and the sympathetic neurone blocking drug, guanethidine, respectively. Chemical denervation with 6-hydroxydopamine abolished the inhibitory effect of guanethidine. Simultaneous treatment with atropine and guanethidine did not abolish the response to nicotine, but the degree of inhibition was comparable to that obtained with atropine alone.

3 The nicotine-induced contraction was insensitive to bunazosin and yohimbine (α_1- and α_2-adrenoceptor antagonists, respectively), and exogenously applied noradrenaline did not cause a contraction even in the presence of blockade of noradrenaline uptake mechanisms with desipramine and normetanephrine and of β-adrenoceptors with propranolol, suggesting a non-adrenergic nature of the sympathomimetic effect of nicotine in this tissue.

4 The nicotine-induced contraction in the presence of atropine was abolished after desensitization of P_2-purinoceptors with α, β-methylene adenosine 5'-triphosphate, a slowly degradable ATP analogue selective for P_2-purinoceptors. By this desensitization, the response to ATP, but not to histamine, was also abolished.

5 A cyclo-oxygenase inhibitor flurbiprofen partially inhibited the nicotine-induced contraction. The degree of the inhibition was more pronounced in the presence of atropine than in its absence. Flurbiprofen antagonized the response to exogenously applied ATP in an unsurmountable manner, but not that to carbachol.

6 The present results suggest that nicotine might induce a contraction through an interaction with nicotinic receptors located on the terminals of, possibly, (i) parasympathetic cholinergic, (ii) sympathetic non-adrenergic and (iii) non-sympathetic purinergic nerves in guinea-pig detrusor preparations, and that a portion of the contraction due to the purine nucleotide released is possibly potentiated by intramural prostaglandin(s). Parasympathetic cholinergic output might be modulated by an unknown excitatory substance released by nicotine from sympathetic nerve.

7 Nicotine reveals a latent excitatory effect of the sympathetic hypogastric nerve which innervates guinea-pig detrusor.

Introduction

Nicotine has been reported to induce a tetrodotoxin (TTX)-resistant response in many peripheral organs. In isolated preparations innervated by sympathetic nerves, such as rabbit pulmonary artery (Su & Bevan, 1970), rat vas deferens, cat splenic strip and guinea-pig atrium (Jayasundar & Vohra, 1978), and rabbit and guinea-pig aorta (Ikushima *et al.*, 1981; 1982), nicotine has been considered to act on presynaptic nicotinic receptors (Trendelenburg, 1965; Su & Bevan, 1970; Jayasundar & Vohra, 1978; Ikushima *et al.*, 1981; 1982). On the other hand, nicotine produces a parasympathomimetic effect through a TTX-resistant release of acetylcholine in bronchial tissues of several animal species (Takayanagi *et al.*,

[1] Author for correspondence.

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1984; Kizawa *et al.*, 1988; Takayanagi *et al.*, 1988). It has also been reported that nicotine releases tachykinin(s) from chemosensitive sensory nerve in guinea-pig bronchus (Kizawa & Takayanagi, 1985) and rabbit iris sphincter (Hisayama *et al.*, 1988). These results suggest that nicotinic receptors might be located at sites other than the autonomic ganglia and that the drug may release substance(s) other than acetylcholine or noradrenaline.

The urinary tract receives parasympathetic, sympathetic, sensory and so-called non-cholinergic, non-adrenergic innervations (El Badawi & Schenk, 1966; Taira, 1972; Burnstock *et al.*, 1978; Alm *et al.*, 1978; Hökfelt *et al.*, 1978). They present a complex picture in terms of excitatory innervation. Histochemical studies revealed the existence of dopamine-β-hydroxylase-positive fibres (Hökfelt *et al.*, 1978), but their physiological significance in micturition is obscure (De Groat & Booth, 1980). Burnstock *et al.* (1978) have proposed that non-cholinergic, non-adrenergic nerves may be purinergic.

The retention of radioactive nicotine in the urinary bladder wall still persisted 1 month after intravenous administration of nicotine (Szüts *et al.*, 1978). Such a long-term accumulation of nicotine might be important pharmacologically and pathophysiologically. We have found more recently that responsiveness to nicotine in rabbit detrusor strips was reduced following chronic nicotine treatment (Kizawa *et al.*, 1988). Similar tolerance to the central effects of nicotine after chronic nicotine treatment has been reported (Hubbard & Gohd, 1975; Marks *et al.*, 1983; Yamanaka *et al.*, 1985). The detailed mechanism of action of nicotine, however, has not as yet been investigated.

Therefore, in the present study, we attempted to delineate the mechanisms of action of nicotine, in guinea-pig isolated detrusor strips.

Methods

Female Hartley strain guinea-pigs weighing between 300 and 500 g were reared on a standard diet and given tap water to drink.

The guinea-pigs were killed and the urinary bladder was rapidly removed; a longitudinal strip (about 2 mm × 20 mm) of the detrusor muscle was prepared.

Each strip was suspended vertically under a resting load of 1 g in an organ bath which contained Krebs solution of the following composition (mM): NaCl 118, KCl 4.75, $CaCl_2$ 2.50, $MgSO_4$ 1.20, KH_2PO_4 1.20, $NaHCO_3$ 25.0 and glucose 10.0. The organ bath was maintained at 37°C and constantly gassed with carbogen (95% O_2 + 5% CO_2). The responses to drugs were recorded isotonically.

The experiments were started after the preparation had been allowed to equilibrate for about 60 min. After priming twice or three times with 300 nM carbachol, the first control dose of nicotine (1 mM) was applied. The drug was thoroughly washed out as soon as the contractile response had reached a maximum, to avoid development of desensitization. After 90-min incubation with the appropriate treatments, the second test dose of nicotine was applied.

When a contraction was induced by potassium depolarization, isotonic 120 mM K-solution was used. The isotonic K-solution was prepared by increasing the potassium concentration of the normal solution to 120 mM and reducing the sodium concentration correspondingly.

Chemical denervation *in vitro* of sympathetic nerve with 6-hydroxydopamine (6-OHDA) followed the method of Aprigliano & Hermsmeyer (1976). Briefly, the preparation was continuously perfused with unbuffered electrolyte solution ($NaHCO_3$ and KH_2PO_4 were omitted from the Krebs solution) containing 1.78 mM (300 μg ml^{-1}) 6-OHDA and 20 μM glutathione for 20 min. During the 6-OHDA treatment, the detrusor was not gassed with carbogen.

Electrical field stimulation was applied through a pair of platinum-wire electrodes. Stimulus parameters were 0.5 ms duration, frequencies of 1 to 30 Hz and supramaximum voltage for 2 s.

Desensitization to α, β-methylene adenosine 5'-triphosphate (MeATP) was produced by the method of Kasakov & Burnstock (1983).

All numerical data are expressed as means ± s.e., and the number of experiments is shown in parentheses. The pD_2 value for a drug (negative logarithm of molar concentration which produced 50% of its maximum response) was calculated by graphical analysis. The pD'_2 value was determined by the method of van Rossum (1963). Statistical analyses were performed by use of Student's t test and Duncan's new multiple range test as appropriate. A P value of <0.05 was considered a significant difference.

Drugs used were nicotine bitartrate (Nakarai Chemicals, Ltd., Kyoto, Japan), carbachol chloride, atropine sulphate, desipramine hydrochloride, normetanephrine hydrochloride, propranolol hydrochloride, ATP, MeATP, glutathione and 6-OHDA hydrobromide (Sigma Chemical Co., MO., USA), physostigmine salicylate (E. Merck AG, Darmstadt, FDR), hexamethonium dibromide (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan), TTX (Sankyo Co., Ltd., Tokyo, Japan), histamine dihydrochloride, noradrenaline bitartrate and yohimbine hydrochloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Flurbiprofen, guanethidine sulphate and bunazosin hydrochloride were donated by Kaken

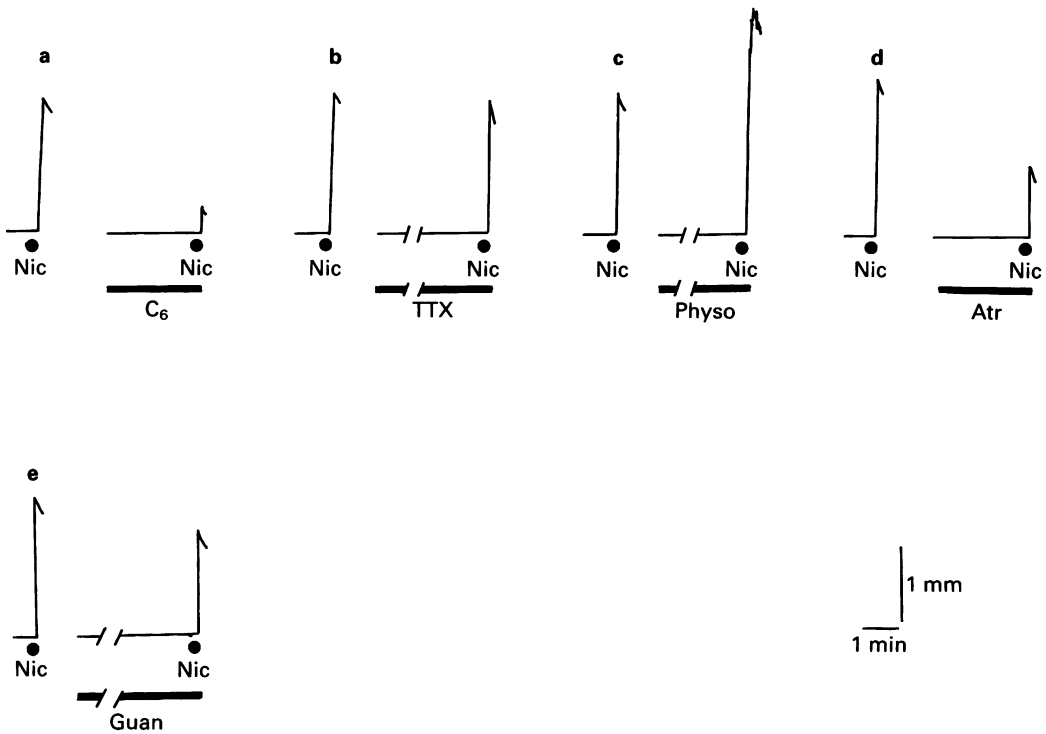

Figure 1 Typical tracings illustrating effects of some drugs on the nicotine-induced contraction of guinea-pig detrusor strips. (a) Hexamethonium (C_6) (0.1 mM); (b) tetrodotoxin (TTX) (3 μM); (c) physostigmine (Physo) (0.1 μM); (d) atropine (Atr) (1 μM); (e) guanethidine (Guan) (3 μM). Dots indicate application of 1 mM nicotine (Nic). Nicotine was washed out as soon as the contractile response had reached a maximum. Solid lines indicate treatment with drugs. The vertical and horizontal scales are the shortening of the strip in mm and time in min, respectively. The preparation was treated with each drug for 15 min (TTX), 30 min (Physo, Guan) or 5 min (the other drugs). One of 6 to 8 experiments is shown.

Pharmaceutical Co., Ltd. (Tokyo, Japan), Ciba-Geigy (Japan), Ltd. (Hyogo, Japan) and Eisai Co., Ltd. (Tokyo, Japan), respectively. All drugs were in powder form and they were dissolved in deionized and distilled water. Other chemicals used were of analytical grade.

Results

Nicotine induced only a phasic contraction in the isolated detrusor muscle of guinea-pig (Figure 1). A concentration-response curve for nicotine was obtained at concentrations between 30 μM and 3 mM (Figure 2). The response to 3 mM nicotine was 61.9 $\pm$ 9.6% ($n = 6$) of that to isotonic 120 mM K-solution.

The contractile response to a nearly maximum concentration of nicotine (1 mM) was greatly reduced by 0.1 mM hexamethonium (Figure 1a), and resistant to 3 μM TTX (Figure 1b). The response was potentiated by 0.1 μM physostigmine (Figure 1c) and partially inhibited to 50% by 1 μM atropine (Figure 1d).

The nicotine-induced contraction was also reduced to about 70% of the control by 3 μM guanethidine (Figure 1e). An increase in the concentration of guanethidine up to 10 μM was not accompanied by further reduction of the nicotine-induced contraction, nor did guanethidine (3 μM) have any effect on the concentration-response curve for carbachol (Figure 3a). The pD_2 values for carbachol in the control and the treated preparations were 6.04 $\pm$ 0.07 and 6.06 $\pm$ 0.05, respectively, and the maximum response in the presence of guanethidine was 101.9 $\pm$ 0.8% of controls ($n = 6$): the difference between the pD_2 values or the maximum responses was not significant. When the preparation was chemically denervated by 6-OHDA, inhibition of the nicotine-induced contraction by guanethidine was abolished: percentage inhibition was reduced from 33.4 $\pm$ 4.7% ($n = 8$) to 15.0 $\pm$ 6.9% ($n = 6$), the latter value was not significantly different from zero

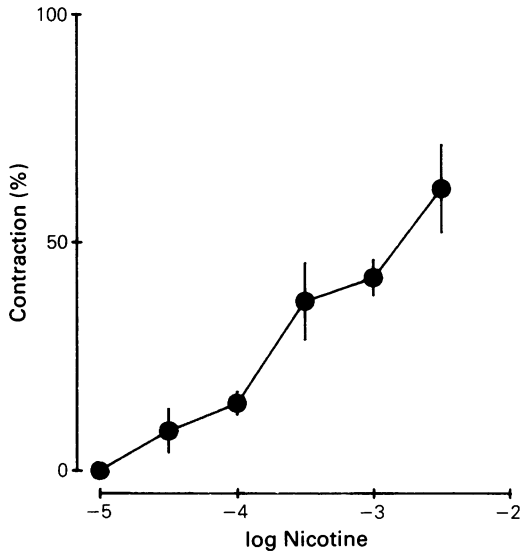

Figure 2 Concentration-response curves for nicotine in guinea-pig detrusor strips. Abscissa scale: log molar concentration of nicotine. Ordinate scale: % of contraction by isotonic 120 mM K-solution. Each value is presented as a mean with s.e. (vertical bar) of 6 experiments.

inhibition. Guanethidine (3 μM) had no effect on the excitatory response to electrical field stimulation at the frequencies examined (1 to 30 Hz). The nicotine-induced contraction was not influenced by bunazosin or yohimbine (Table 1). Further, exogenously applied noradrenaline up to at least 100 μM did not cause any response in the guinea-pig detrusor in the presence of the noradrenaline uptake blockers desipramine (100 nM) and normetanephrine (1 μM) and of the β-adrenoceptor blocker propranolol (1 μM) in combination. Simultaneous treatment with atropine and guanethidine did not abolish the response to nicotine (Table 1).

Table 1 Effects of drugs on the contractile responses to nicotine in the absence and presence of atropine (1 μM) in guinea-pig detrusor strips

Treatment	*% of contraction*	
Nicotine, 1 mM	100	
+ hexamethonium, 100 μM (5 min)	31.3 ± 4.4*	(6)
+ tetrodotoxin, 3 μM (15 min)	95.0 ± 2.9	(6)
+ physostigmine, 0.1 μM (30 min)	150.3 ± 9.7*	(6)
+ atropine, 1 μM (5 min)	49.5 ± 4.7*	(6)
+ guanethidine, 3 μM (30 min)	67.6 ± 4.7*	(8)
+ bunazosin, 0.1 μM (5 min)	101.8 ± 4.9	(6)
+ yohimbine, 0.3 μM (5 min)	101.6 ± 2.9	(6)
+ atropine, 1 μM (5 min) and guanethidine, 3 μM (30 min)	44.4 ± 5.5*	(6)
+ methysergide, 1 μM (5 min)	94.7 ± 7.6	(6)
+ flurbiprofen, 1 μM (1 h)	87.9 ± 4.6*	(6)
Nicotine, 1 mM in the presence of atropine, 1 μM (5 min)	100	
+ flurbiprofen, 1 μM (1 h)	47.9 ± 8.2*†	(6)

* Significant difference from 100% at $P < 0.05$.
† Significant difference from the corresponding value obtained in the absence of atropine.

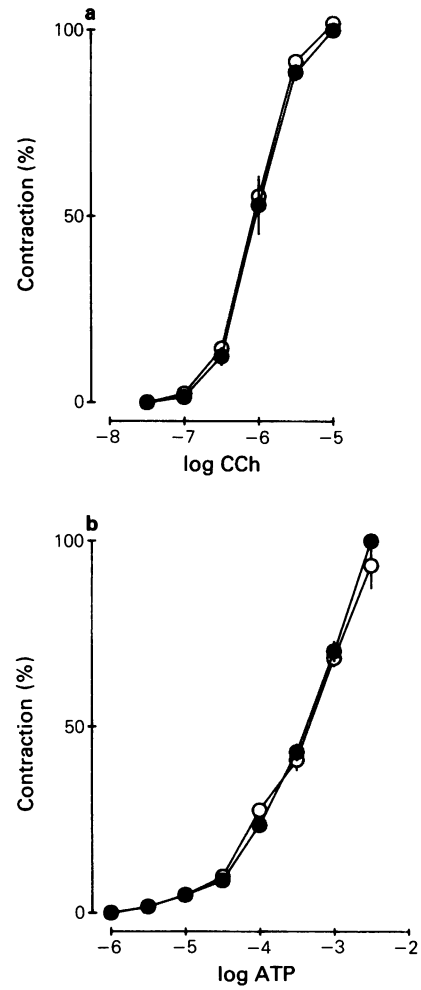

Figure 3 Effects of guanethidine on the concentration-response curves for carbachol (a) and ATP (b) in guinea-pig detrusor strips: (●) control; (○) with 3 μM guanethidine. Abscissa scale: log molar concentration of drugs. Ordinate scale: % of contraction induced by 30 μM carbachol (CCh) (a) or 3 mM ATP (b). Each value is presented as a mean with s.e. (vertical bar) of 6 experiments.

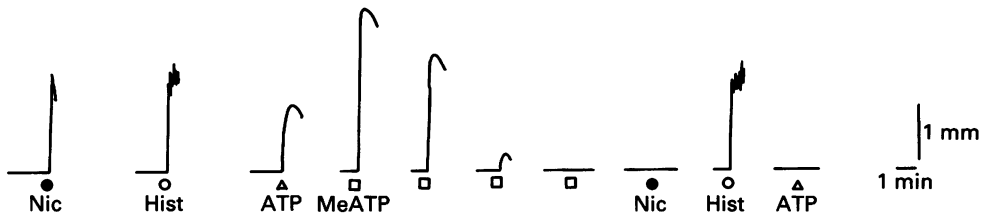

Figure 4 Typical tracings illustrating effect of α, β-methylene ATP (MeATP) desensitization of the response to 1 mM nicotine (●; Nic), 50 μM ATP (△; ATP) and 1 μM histamine (○; Hist) in the presence of 1 μM atropine. MeATP desensitization was achieved by 4 to 6 (4 times in this example) succesive applications (□) at approximately 4 min intervals. The vertical and horizontal scales are the shortening of the strip in mm and time in min, respectively. One of 6 experiments is shown.

These results suggest that parasympathetic cholinergic (atropine-sensitive), sympathetic non-adrenergic (guanethidine-sensitive) and non-cholinergic, non-sympathetic (resistant to both atropine and guanethidine) components may contribute to the nicotine-induced contractile response. The degree of inhibition by simultaneous treatment with atropine and guanethidine was, however, comparable to that obtained with atropine alone (Table 1), suggesting some interaction between cholinergic and sympathetic non-adrenergic components. A 5-hydroxytryptamine receptor antagonist methysergide (1 μM) had no effect on the nicotine-induced contraction (Table 1).

The following experiments with MeATP were carried out in the presence of 1 μM atropine to determine the mediator released other than acetylcholine. Application of MeATP (50 μM) elicited a rapid phasic contraction. With successive applications of MeATP for 20 s at 2–4 min intervals, desensitization of the drug-induced response was achieved. The desensitization was progressive, and after 4–6 periods of application the excitatory responses were completely abolished (Figure 4), as already reported by Kasakov & Burnstock (1983).

In addition, the responses to nicotine and ATP (100 μM), but not to histamine (1 μM), were completely abolished after desensitization to MeATP had been achieved (Figure 4 and Table 2).

Guanethidine (3 μM) had no effect on the concentration-response curve for ATP (Figure 3b). The pD_2 values for ATP in the control and the treated preparations were 3.38 ± 0.03 and 3.31 ± 0.05, respectively, and the maximum response in the presence of guanethidine was $93.4 \pm 6.0\%$ of controls ($n = 6$): the difference between the pD_2 values or the maximum responses was not significant.

It has been reported that ATP, whilst acting on the P_2-purinoceptors which mediate ATP responses, induces prostaglandin synthesis (Brown & Burnstock, 1981; Burnstock & Kennedy, 1985). A potent cyclo-oxygenase inhibitor flurbiprofen partially inhibited the response to nicotine. The degree of the inhibition was more pronounced in the presence of atropine than in its absence (Table 1). Although flurbiprofen had no effect on the response to carbachol (Figure 5a), the drug antagonized the response to exogenously applied ATP in an unsurmountable manner (Figure 5b). The pD_2 values for carbachol in the control and the treated preparations were 5.84 ± 0.03 and 5.80 ± 0.02 ($n = 4$), and those for ATP were 3.56 ± 0.09 and 3.70 ± 0.14 ($n = 6$), respectively. In either case, the difference between the pD_2 values was not significant. On the other hand, the maximum responses to carbachol and ATP in the presence of flurbiprofen were 100.6 ± 1.1 ($n = 4$) and $73.2 \pm 6.2\%$ ($n = 6$) of the respective controls: only the maximum response to ATP was significantly reduced. The pD'_2 value of flurbiprofen was 5.70 ± 0.12 ($n = 6$).

Table 2 Effects of desensitization of P_2-purinoceptors with α,β-methylene ATP (MeATP) on the contractile responses to nicotine in the atropinized (1 μM) guinea-pig detrusor strips

Treatment	*% of contraction*	
Isotonic 120 mM K-solution	100	
Nicotine, 1 mM in the presence of		
atropine, 1 μM (5 min)	34.7 ± 5.4	(6)
+ MeATP, 50 μM	0.0 ± 0.0*	(6)
ATP, 100 μM in the presence of		
atropine, 1 μM (5 min)	20.3 ± 5.6	(6)
+ MeATP, 50 μM	0.0 ± 0.0*	(6)
Histamine, 1 μM in the presence of		
atropine, 1 μM (5 min)	38.9 ± 4.6	(6)
+ MeATP, 50 μM	38.0 ± 4.3	(6)

*Significant difference from the corresponding value obtained in strips not treated with MeATP (control) at $P < 0.05$.
Desensitization with MeATP was carried out as illustrated in Figure 4.

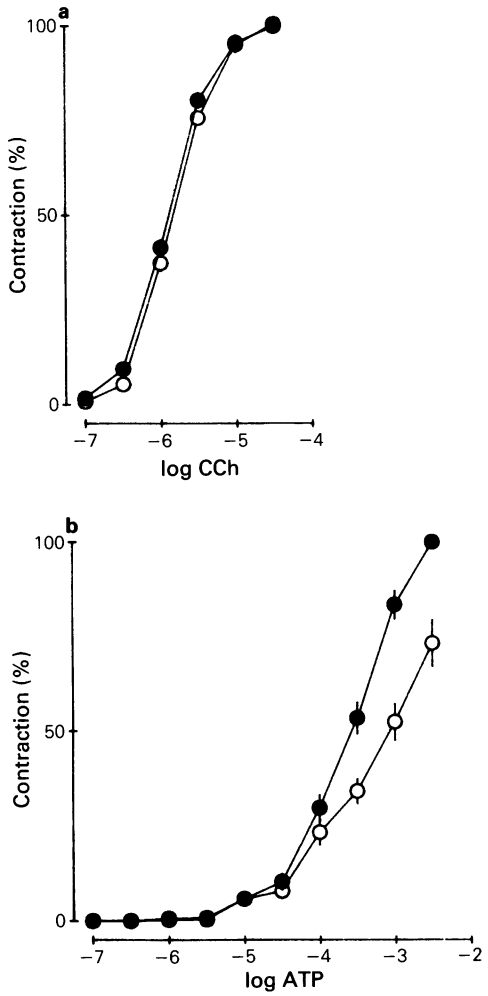

Figure 5 Effects of flurbiprofen on the concentration-response curves for carbachol (a) and ATP (b) in guinea-pig detrusor strips: (●) control; (○) with 1 μM flurbiprofen. Abscissa scale: log molar concentration of drugs. Ordinate scale: % of contraction induced by 30 μM carbachol (CCh) (a) or 3 mM ATP (b). Each value is presented as a mean with s.e. (vertical bar) of 4 (a) or 6 (b) experiments.

Discussion

Nicotine induced a transient contraction in the guinea-pig detrusor strips in this study. This contractile response was greatly reduced by hexamethonium, indicating that the effect of nicotine resulted from interaction with autonomic nicotinic receptors.

On the other hand, the contractile response to nicotine was not influenced by TTX. It is well known that nicotine acts on nicotinic receptors located on the autonomic ganglion to induce generation and propagation of TTX-sensitive sodium action potential along the postganglionic nerve fibres, and that these impulses reaching nerve terminals cause release of a neurotransmitter. However, in some tissues, TTX has been reported not to inhibit the response to nicotine. These findings suggest that two mechanisms are involved in the transmitter release induced by nicotine, one dependent on sodium action potentials and the other independent (Trendelenburg, 1965; Su & Bevan, 1970; Jayasundar & Vohra, 1978; Ikushima *et al.*, 1981; 1982; Takayanagi *et al.*, 1984; 1988; Kizawa & Takayanagi, 1985). In the guinea-pig detrusor, the contractile response to nicotine seems to be produced mainly through the latter mechanism, and the nicotinic receptors involved are probably located on the nerve terminals rather than on the ganglion cells (Hisayama *et al.*, 1988).

Potentiation by physostigmine and partial blockade by atropine of the nicotine-induced contraction strongly suggest that part of the contractile response to nicotine in guinea-pig detrusor is cholinergic and the remaining portion is non-cholinergic in nature. These results are fairly consistent with those obtained by electrical field stimulation (Westfall *et al.*, 1983; MacKenzie & Burnstock, 1984; Callahan & Creed, 1986). Callahan & Creed (1986) reported that atropine reduced the response evoked by electrical field stimulation at all frequencies to about 60%.

On the other hand, the contractile response to nicotine was also partially inhibited by guanethidine. It seems unlikely that this effect of guanethidine was nonspecific, since chemical denervation with 6-OHDA abolished the inhibitory effect of guanethidine, guanethidine did not inhibit the muscarinic and purinergic (see below) receptor mechanisms, and further, in contrast to nicotine, the response to electrical field stimulation was resistant to the drug. These findings also suggest that nicotine might reveal a latent sympathetic nerve activity in the guinea-pig detrusor which releases an excitatory transmitter or modulator. The nicotine-induced contraction was not antagonized by bunazosin or yohimbine and exogenously applied noradrenaline caused no response even when noradrenaline uptake mechanisms and β-adrenoceptors were blocked. Consequently, noradrenaline seems not to play a major role, if any, in the sympathetic component as a transmitter or a modulator.

Many researchers have produced evidence that 'non-cholinergic, non-adrenergic nerves' may innervate this tissue (e.g., Taira, 1972; Burnstock *et al.*, 1978). With regard to this proposal and our present findings, the following points should be noted.

Whereas the 'non-cholinergic, non-adrenergic' contraction reported by other investigators has been obtained in the presence of atropine and guanethidine, the non-adrenergic nicotine action described above was blocked by guanethidine. Therefore, for simplicity, we refer to properties resistant to adrenoceptor blockers and guanethidine, as non-adrenergic and non-sympathetic, respectively. With these definitions in mind, in addition to a sympathetic non-adrenergic contraction, nicotine elicits a non-cholinergic, non-adrenergic contraction possibly via a non-cholinergic, non-sympathetic nerve, because some contractile component remained after treatment with atropine and guanethidine.

The degree of inhibition of nicotine-induced contraction was, however, much the same in preparations treated with atropine alone and atropine plus guanethidine: in other words, it would be possible to say that atropine diminishes the effect of guanethidine. This phenomenon cannot be accounted for by the idea that guanethidine inhibits the muscarinic receptor mechanism like atropine, since guanethidine had no effect on the concentration-response curve for carbachol. Similar results were obtained in rabbit detrusor strips (our unpublished observation). One possible explanation is that a non-adrenergic unknown substance released from sympathetic nerve may not act on muscle cells directly, but may modulate the activity of parasympathetic nerve in an excitatory manner, to augment the acetylcholine output. In such a scheme, it is possible that when muscarinic receptors on the detrusor are blocked by atropine, the effect of guanethidine would not be observed since the cholinergic response would be totally abolished even if acetylcholine output was increased via sympathetic nerve activity.

The present study demonstrates that after desensitization to MeATP, the atropine-resistant contraction induced by nicotine was completely abolished. This finding is similar to that obtained by electrical field stimulation, although in the latter case, the sympathetic component is not observed (Ambache & Zar, 1970; Burnstock *et al.*, 1978; Fujii, 1988). MeATP is a highly selective P_2-purinoceptor agonist (Burnstock & Kennedy, 1985), and persistent treatment finally desensitizes the P_2-purinoceptor itself in many preparations (Kasakov & Burnstock, 1983; Katsuragi & Furukawa, 1985). Electrophysiological studies also demonstrate that changes in the membrane potential and conductance induced by MeATP are transient and return to control values during continuous exposure to this agent (Fujii, 1988).

Since the ATP-induced contraction, but not the histamine-induced contraction, was abolished by P_2-purinoceptor desensitization, which is in agreement with the previous report of Kasakov & Burnstock (1983), the desensitization of the atropine-resistant response to nicotine is probably restricted to the purinoceptors. These results strongly suggest that a purine nucleotide is the transmitter involved in non-cholinergic, non-sympathetic nicotine action.

ATP has been known to stimulate prostaglandin synthesis in many preparations (Brown & Burnstock, 1981; Burnstock & Kennedy, 1985). This is consistent with our results that exogenously applied ATP-induced contraction was unsurmountably inhibited by the potent cyclo-oxygenase inhibitor flurbiprofen. It is assumed that flurbiprofen did not behave as a general depressant of muscular reactivity, since carbachol-induced contraction was totally resistant to the drug. It is probable that the purine nucleotide released by nicotine from non-cholinergic, non-sympathetic nerve, whilst acting on the P_2-purinoceptors to contract the detrusor directly, indirectly potentiates the response to the nucleotide itself via induction of prostaglandin synthesis. Burnstock *et al.* (1978) reported that prostaglandin E_1, (PGE_1), PGE_2 and $PGF_{2\alpha}$ induced weak slow contractions, which differ from the rapid nicotine-induced contraction observed in this study, and that the response to ATP was potentiated in the presence of such prostaglandins.

The less pronounced inhibition by flurbiprofen of the nicotine-induced contraction in the absence of atropine than in its presence may be due to an overwhelming stimulation by acetylcholine which partially obscures the purinergic portion potentiated by prostaglandin(s). The possibility remains, however, that prostaglandin(s) might additionally act as a neuromodulator.

In conclusion, the present studies suggest that nicotine induces contraction through an interaction with nicotinic receptors located on the terminals of, possibly, (i) parasympathetic cholinergic, (ii) sympathetic non-adrenergic and (iii) non-sympathetic purinergic nerves in guinea-pig detrusor strips, and that a portion of the contraction due to the purine nucleotide released is possibly potentiated by intramural prostaglandin(s). Parasympathetic cholinergic output might be modulated by an unknown excitatory substance released from the sympathetic hypogastric nerve, the latent activity of which would be revealed by nicotine.

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References

ALM, P., ALUMETS, J., BRODIN, E., HÅKANSON, R., NILSSON, G., SJÖBERG, N.-O. & SUNDLER, F. (1978). Peptidergic (substance P) nerves in the genito-urinary tract. *Neuroscience*, **3**, 419-425.

AMBACHE, N. & ZAR, M.A. (1970). Non-cholinergic transmission by postganglionic motor neurones in the mammalian bladder. *J. Physiol.*, **210**, 761–783.

APRIGLIANO, O. & HERMSMEYER, K. (1976). *In vitro* denervation of the portal vein and caudal artery of the rat. *J. Pharmacol. Exp. Ther.*, **198**, 568–577.

BROWN, C.M. & BURNSTOCK, G. (1981). The structural conformation of the polyphosphate chain of the ATP molecule is critical for its promotion of prostaglandin biosynthesis. *Eur. J. Pharmacol.*, **69**, 81–86.

BURNSTOCK, G., COCKS, T., CROWE, R. & KASAKOV, L. (1978). Purinergic innervation of the guinea-pig urinary bladder. *Br. J. Pharmacol.*, **63**, 125–138.

BURNSTOCK, G. & KENNEDY, C. (1985). Is there a basis for distinguishing two types of P_2-purinoceptor? *Gen. Pharmacol.*, **16**, 433–440.

CALLAHAN, S.M. & CREED, K.E. (1986). Non-cholinergic neurotransmission and the effects of peptides on the urinary bladder of guinea-pigs and rabbits. *J. Physiol.*, **374**, 103–115.

DE GROAT, W.C. & BOOTH, A.M. (1980). Inhibition and facilitation in parasympathetic ganglia of the urinary bladder. *Fed. Proc.*, **39**, 2990–2996.

EL BADAWI, A. & SCHENK, E.A. (1966). Dual innervation of the mammalian urinary bladder: a histochemical study of the distribution of cholinergic and adrenergic nerves. *Am. J. Anat.*, **119**, 405–417.

FUJII, K. (1988). Evidence for adenosine triphosphate as an excitatory transmitter in guinea-pig, rabbit and pig urinary bladder. *J. Physiol.* (in press).

HISAYAMA, T., SHINKAI, M., TAKAYANAGI, I., MORIMOTO, S.-I. & TOYOTO, T. (1988). Mechanism of action of nicotine in isolated iris sphincter preparations of rabbit. *Br. J. Pharmacol.*, **95**, 465–472.

HÖKFELT, T., SCHULTZBERG, M., ELDE, R., NILSSON, G., TERENIUS, L., SAID, S. & GOLDSTEIN, M. (1978). Peptide neurons in peripheral tissues including the urinary tract: immunohistochemical studies. *Acta Pharmacol. Toxicol.*, **43**, 79–89.

HUBBARD, J.E. & GOHD, R.S. (1975). Tolerance development to the arousal effects of nicotine. *Pharmacol. Biochem. Behav.*, **3**, 471–476.

IKUSHIMA, S., MURAMATSU, I. & FUJIWARA, M. (1981). Effects of 4-aminopyridine on the adrenergic nerve terminals. *J. Pharmacol. Exp. Ther.*, **219**, 792–797.

IKUSHIMA, S., MURAMATSU, I. & FUJIWARA, M. (1982). Nicotine-induced response in guinea-pig aorta enhanced by goniopora toxin. *J. Pharmacol. Exp. Ther.*, **223**, 790–794.

JAYASUNDAR, S. & VOHRA, M.M. (1978). Mechanism of nicotine-induced release of norepinephrine from adrenergic nerve endings: is generation and propagation of impulses necessary? *Arch. Int. Pharmacodyn.*, **232**, 202–210.

KASAKOV, L. & BURNSTOCK, G. (1983). The use of the slowly degradable analog, α, β-methylene ATP, to produce desensitization of the P_2-purinoceptor: effect on non-adrenergic, non-cholinergic responses of the guinea-pig urinary bladder. *Eur. J. Pharmacol.*, **86**, 291–294.

KATSURAGI, T. & FURUKAWA, T. (1985). Novel, selective purinoceptor antagonist: investigation of ATP as a neurotransmitter. *Trends Pharmacol. Sci.*, **6**, 337–339.

KIZAWA, Y. & TAKAYANAGI, I. (1985). Mechanism of action of nicotine on crab-eating monkey isolated bronchial smooth muscle. *Can. J. Physiol. Pharmacol.*, **63**, 1471–1473.

KIZAWA, Y., TAKAYANAGI, I. & SHINKAI, M. & OHNO, Y. (1988). Pharmacological action of nicotine in the isolated urinary bladder from rabbit: special reference to the chronic nicotine treatment. *Gen. Pharmacol.*, **19**, 269–271.

MACKENZIE, I. & BURNSTOCK, G. (1984). Neuropeptide action on the guinea-pig bladder; a comparison with the effects of field stimulation and ATP. *Eur. J. Pharmacol.*, **105**, 85–94.

MARKS, M.J., BURCH, J.B. & COLLINS, A.C. (1983). Effects of chronic nicotine infusion on tolerance development and nicotine receptors. *J. Pharmacol. Exp. Ther.*, **226**, 817–825.

SU, C. & BEVAN, J.A. (1970). Blockade of the nicotine-induced norepinephrine release by cocaine, phenoxybenzamine and desipramine. *J. Pharmacol. Exp. Ther.*, **175**, 533–540.

SZÜTS, T., OLSSON, S., LINDQUIST, N.G. & ULLBERG, S. (1978). Long-term fate of [^{14}C]nicotine in the mouse: retention in the bronchi, melanin-containing tissues and urinary bladder wall. *Toxicol.*, **10**, 207–220.

TAIRA, N. (1972). The autonomic pharmacology of the bladder. *Ann. Rev. Pharmacol.*, **12**, 197–208.

TAKAYANAGI, I., KIZAWA, Y. & HIRUTA, T. (1984). Tetrodotoxin-resistant response to nicotine in rabbit bronchial preparation. *Eur. J. Pharmacol.*, **104**, 351–356.

TAKAYANAGI, I., KIZAWA, Y., TOYODA, T. & FURUKAWA, A. (1988). Characterization of nicotine-induced contraction in the canine bronchus. *Comp. Biochem. Physiol.*, **89C**, 11–13.

TRENDELENBURG, U. (1965). Nicotine and the peripheral autonomic nervous system. In *Tobacco Alkaloids and Related Compounds*, ed. von Euler, U.S. pp. 167–177, New York: Pergamon Press.

VAN ROSSUM, J.M. (1963). Cumulative dose-response curves in isolated organs and the evaluation of drug parameters. *Arch. Int. Pharmacodyn.*, **143**, 299–330.

WESTFALL, D.P., FEDAN, J.S., COLBY, J., HOGABOOM, G.K. & O'DONNELL, J.P. (1983). Evidence for a contribution by purine to the neurogenic response of the guinea-pig urinary bladder. *Eur. J. Pharmacol.*, **87**, 415–422.

YAMANAKA, K., OSHITA, M. & MURAMATSU, I. (1985). Alteration of alpha and muscarinic receptors in rat brain and heart following chronic nicotine treatment. *Brain Res.*, **348**, 241–248.

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