Br. J. Pharmacol. (1991), **103**, 1191–1195

Facilitatory effects of tachykinins and guanethidine on the acetylcholine output stimulated by nicotine from guinea-pig bladder

Michiko Shinkai, [1]Issei Takayanagi & Teruko Kato

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

1 Contractile responses and acetylcholine release evoked by nicotine in guinea-pig detrusor strips were determined by isotonic transducer and radioimmunoassay, respectively. Nicotine stimulated acetylcholine release and a contractile response in guinea-pig detrusor strips treated with the cholinesterase inhibitor, methanesulphonyl fluoride (MSF). Both actions evoked by nicotine were antagonized by the nicotinic receptor antagonist, hexamethonium but were insensitive to tetrodotoxin.

2 A sympathetic nerve blocker, guanethidine and a tachykinin antagonist, [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]-substance P (rpwwL-SP) partially inhibited the acetylcholine release evoked by nicotine to much the same degree. The inhibitory effects of guanethidine and rpwwL-SP on acetylcholine release were significantly greater than corresponding effects on the contraction evoked by nicotine.

3 In preparations treated with rpwwL-SP to block the tachykinin receptors, guanethidine had no effect on the response to nicotine. Conversely, after treatment with guanethidine to block release of a mediator from sympathetic nerve endings, nicotine-induced responses were not affected by rpwwL-SP.

4 Nicotine-induced contraction was reduced to 30% by the muscarinic cholinoceptor antagonist, atropine and completely abolished after desensitization of P_2-purinoceptors with α,β-methylene ATP in the presence of atropine.

5 A concentration-contractile response curve to neurokinin A (NKA) was shited to the left after cholinesterase inhibition with MSF. Atropine abolished the facilitatory effect of MSF and partially inhibited contractions induced by NKA at 100 nM to 1 μM. The contractile responses to substance P methyl ester (SPOMe) and Tyr^0-neurokinin B (Tyr^0-NKB) were not influenced by MSF or atropine.

6 After desensitization of NK_1 tachykinin receptors with SPOMe or preincubation with senktide, the cholinergic component of the nicotine-induced contraction was the same as the control value (100%).

7 Our findings give further support to our previous results: nicotine stimulates acetylcholine release in a tetrodotoxin-resistant manner in guinea-pig bladder and acetylcholine release evoked by nicotine is increased by the coordinated action of sympathetic nerves and tachykinin(s). It is suggested that the tachykinin receptor subtype involved in acetylcholine release is NK_1.

Keywords: Guinea-pig bladder; nicotine; radioimmunoassay; acetylcholine release; sympathetic nerve; tachykinin(s)

Introduction

Substance P (SP)-like immunoreactivity (SP-LI) and immunoreactive nerves (SP-IR nerves) have been identified throughout the bladder (Alm *et al.*, 1978; Hokfelt *et al.*, 1978). SP causes contraction of the guinea-pig bladder (Falconieri Erspamer *et al.*, 1980; Hunter & Maggio, 1984) and functional NK_1 and NK_2 tachykinin receptor subtypes are present in this tissue (Shinkai & Takayanagi, 1990).

We have shown that nicotine produces a transient, tetrodotoxin-resistant contraction of isolated detrusor strips of guinea-pig, and suggested that the drug might interact with the presynaptic nicotinic receptors located on (1) parasympathetic cholinergic (atropine sensitive), (2) sympathetic non-adrenergic (guanethidine-sensitive, but resistant to bunazosin and yohimbine) and (3) non-sympathetic purinergic (sensitive to desensitization of P_2-purinoceptors by α,β-methylene adenosine 5'-triphosphate) nerves to induce a release of two excitatory final transmitters, acetylcholine and a purine nucleotide (Hisayama *et al.*, 1988a). The potentiation of acetylcholine output from cholinergic neurones by the coordinated action of sympathetic nerve and tachykinin(s) following nicotine stimulation has been shown indirectly through the effect of the tachykinin antagonist [D-Arg^1,D-Pro^2,D-Trp^{7,9}, Leu^{11}]-SP (rpwwL-SP) and the sympathetic nerve blocking agent, guanethidine on the nicotine-induced contraction (Hisayama *et al.*, 1989).

In this study, we determined the role of the sympathetic nerves and tachykinin(s) on nicotine-induced acetylcholine release directly by radioimmunoassay.

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 stunned by a blow on the head and exsanguinated from the femoral artery. The urinary bladder was rapidly removed and 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 a 5 ml 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 response to drugs was 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 (0.1 mM) was applied; after incubation for 60 min with the appropriate treatments, the second test dose of nicotine was applied.

[1] Author for correspondence.

Table 1 Effect of drugs on acetylcholine release and on the contractile responses induced by nicotine in guinea-pig bladder treated wtih methanesulphonyl fluoride (MSF)

Treatment	*Acetylcholine release* (%)	*Contraction* (%)
Nicotine 0.1 mM	100	100
+ hexamethonium, 10 μM	15.6 ± 3.5* (4)	26.1 ± 4.6* (4)
+ tetrodotoxin, 30 μM	112.9 ± 17.9 (4)	97.3 ± 7.7 (4)
+ guanethidine, 3 μM	31.8 ± 9.7* (4)	81.3 ± 2.7* (4)
+ rpwwL-SP, 10 μM	27.6 ± 5.8* (4)	77.6 ± 3.4* (4)
Nicotine, 0.1 mM in the presence of rpwwL-SP, 10 μM	100	100
+ guanethidine, 3 μM	99.5 ± 20.3 (4)	101.5 ± 1.7 (4)
Nicotine, 0.1 mM in the presence of guanethidine, 3 μM	100	100
+ rpwwL-SP, 10 μM	94.6 ± 8.8 (4)	112.7 ± 6.3 (4)

* Significant difference from 100% at $P < 0.05$.
rpwwL-SP: [D-Arg[1],D-Pro[2],D-Trp[7,9],Leu[11]]-substance P.

When we determined acetylcholine release and contractile responses simultaneously, each strip was incubated with 1 mM methanesulphonyl fluoride (MSF) for 30 min in order to inhibit acetylcholinesterase activity irreversibly. After priming twice or three times with 300 nM carbachol, Krebs solution containing various concentrations of drugs were superfused at a rate of 0.4 ml min^{-1} from the bottom and collected by aspiration at the top of an organ bath, and the effects of these drugs on acetylcholine output from the strips as well as contractile responses were studied. The superfusate was collected continuously on ice and divided into fractions for each 3 min period, and then assayed for acetylcholine as described below.

Acetylcholine was determined by radioimmunoassay with rabbit antiserum raised against choline hemiglutarate-bovine serum albumin conjugates and tritiated acetylcholine with a specific activity of 75.1 Ci mmol^{-1} according to the method of Kawashima *et al.* (1988). Assays were performed in duplicate at 4°C. To avoid overestimation by cross-reactivity with nicotine, the standard curve for acetylcholine was derived in the presence of nicotine as appropriate.

A 200 μl portion of the superfusate was incubated overnight with 50 μl of the diluted antiserum (1:350) in Tris-HCl buffer (0.15 M, pH 7.4) containing 0.4% bovine gamma-globulin, 0.05% isofluorophosphate and 50 μl of tritiated acetylcholine (about 12.1 pg, 4500 c.p.m.). The same volume of superfusion fluid containing 0.1 mM nicotine served as a blank. Antibody-bound tritiated acetylcholine was separated from the free tritiated acetylcholine by the ammonium sulphate method (Farr, 1958), and the radioactivity of the precipitates was quantitated in a liquid scintillation counter. Acetylcholine release induced by nicotine was calculated by subtracting the value for spontaneous acetylcholine release from that for the total.

Desensitization to α,β-methylene adenosine 5'-triphosphate (α,β-MeATP) and to substance P methyl ester (SPOMe) was produced by the methods of Kasakov & Burnstock (1983) and Laufer *et al.* (1985), respectively (Figure 3).

The pD_2 value (the negative logarithm of the molar concentration which produced 50% of its maximum responses) for a drug was calculated by graphic analysis. Statistical analyses were performed by Student's *t* test. 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, ATP, MeATP, gamma-globulins, diisopropyl fluorophosphate, bicuculline (Sigma Chemical Co., MO., U.S.A.), hexamethonium dibromide (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan), tetrodotoxin (Sankyo Co., Ltd., Tokyo, Japan), NKA, Tyr^0-NKB, senktide, SPOMe, rpwwL-SP (Peninsula Laboratories, Inc., Belmont, CA, U.S.A.), acetyl[methyl-^{3}H]-choline chloride (Amersham Japan Co., Tokyo, Japan) and methanesulphonyl fluoride (Aldrich Chemical Co., Inc., U.S.A.). Guanethidine sulphate was donated by Ciba-Geigy (Japan), Ltd. (Hyogo, Japan). All drugs used were of analytical grade.

Results

In the isolated detrusor muscle of guinea-pig treated with MSF (1 mM, 30 min) to inhibit acetylcholinesterase irreversibly, a concentration-response curve for the contractile response to nicotine was obtained at concentrations between 10 μM to 30 mM (Figure 1). The pD_2 value for nicotine was 4.21 ± 0.06 ($n = 6$) and the maximum response compared with that to isotonic 120 mM K-solution was 91.6 ± 3.90% ($n = 6$). The contractile response induced by K-solution was not influenced by MSF (95.6 ± 4% of control value, mean of 6 experiments).

Simultaneous measurements of mechanical activity and acetylcholine release

The effects of some drugs on contractile response and acetylcholine release induced by nicotine (0.1 mM) are summarized in Table 1. Acetylcholine release evoked by nicotine (0.1 mM) was 84.9 ± 7.6 pg mg^{-1} tissue per 3 min ($n = 16$). Both the nicotine-induced contraction and acetylcholine release were greatly reduced by 10 μM hexamethonium, and resistant to 3 μM tetrodotoxin (Table 1). The nicotine-induced acetylcholine release was also reduced by 3 μM guanethidine or 10 μM rpwwL-SP to much the same degree (about 30%). On the other hand, the contractile response to nicotine was only weakly reduced by guanethidine or rpwwL-SP (Table 1).

In preparations treated with rpwwL-SP to block the tachykinin receptors, guanethidine had no effect on the responses to

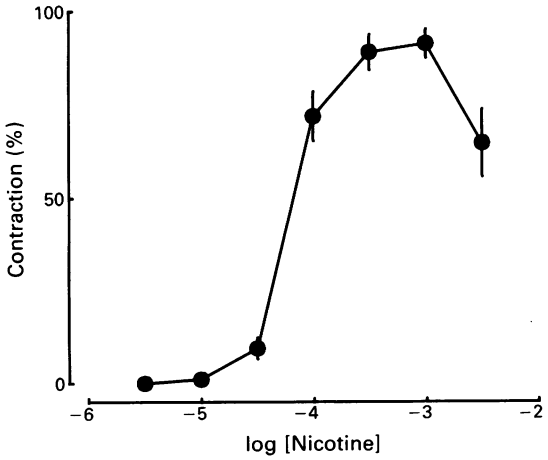

Figure 1 Concentration-response curves for nicotine in guinea-pig detrusor strips treated with methanesulphonyl fluoride (1 mM). Abscissa scale: log molar concentration of nicotine. Ordinate scale: % of contraction induced by isotonic 120 mM K-solution. Each value is presented as a mean with s.e. (vertical line) of 6 experiments.

Table 2 Effects of drugs on the contractile responses to nicotine in guinea-pig bladder treated with methanesulphonyl fluoride

Treatment	*Contraction* (%)
Nicotine, 0.1 mM	100
+ atropine, 1 μM	28.3 ± 4.6* (6)
+ atropine, 1 μM and α,β-MeATP, 50 μM	5.8 ± 3.1*† (6)
+ bunazosin, 0.1 μM	93.7 ± 2.7 (6)
+ yohimbin, 0.3 μM	90.6 ± 5.1 (6)
+ methysergide, 1 μM	97.9 ± 3.7 (6)
+ naloxone, 1 μM	105.6 ± 4.1 (6)
+ bicuculline, 1 μM	100.4 ± 3.8 (6)

* Significant difference from 100% at $P < 0.05$.
† No significant difference from 0% at $P < 0.05$.
α,β-MeATP: α,β-methylene ATP.

nicotine. Conversely, after treatment with guanethidine to prevent release of a mediator from sympathetic nerve endings, the nicotine-induced response was not affected by rpwwL-SP (Table 1).

Effects of some drugs on nicotine-induced contractions in methanesulphonyl fluoride-treated preparations

In the MSF-treated preparation the nicotine-induced contraction was reduced to about 30% of control value by atropine (1 μM). Simultaneous treatment with atropine and α,β-MeATP desensitization to inactivate muscarinic receptors and P_{2X}-purinoceptors respectively, abolished the nicotine contraction. The nicotine-induced contraction was not influenced by pretreatment with bunazosin (1 μM), yohimbine (300 nM), naloxone (1 μM), methysergide (1 μM) or bicuculline (1 μM) (Table 2).

Cholinergic component of the tachykinin-induced contraction

The concentration-response curve for NKA was shifted to the left by treatment with MSF to inhibit acetylcholinesterase activity irreversibly. The pD_2 values for NKA before and after MSF treatment were 6.65 ± 0.11 ($n = 6$) and 7.21 ± 0.03 ($n = 6$), respectively. Atropine (1 μM) abolished the facilitatory effect of MSF and partially inhibited contractions by NKA at 100 nM to 1 μM (Figure 2), suggesting a cholinergic component of the NKA-induced action. In contrast, the pD_2 values of Tyr^0-NKB (water soluble analogue of NKB) and SPOMe were not influenced by MSF or atropine (Table 3). The NK_3 receptor selective agonist, senktide did not cause a contraction in guinea-pig bladder.

Effects of NK_1 and NK_3 selective agonists on the cholinergic component of the nicotine contraction

After desensitization with α,β-MeATP, contractile responses to nicotine (0.1 mM) were not influenced by desensitization by

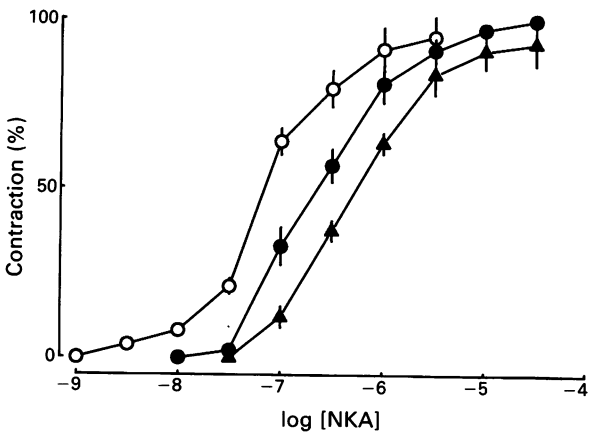

Figure 2 Effect of methanesulphonyl fluoride (MSF) and atropine on the concentration-response curves to neurokinin A (NKA). (●) NKA alone; (○) treated with MSF 1 mM; (▲) in the presence of atropine 1 μM in preparations treated with MSF. Concentration-response curve of NKA was shifted to the left by MSF. Application of atropine abolished the facilitatory effect of MSF and partially inhibited the NKA-induced contraction at 100 nM to 1 μM.

Table 4 Effect of drugs on the contractile responses to nicotine after desensitization with α,β-methylene ATP (α,β-MeATP)

Treatment	*Contraction* (%)
Nicotine, 0.1 mM after desensitization with α,β-MeATP (50 μM)	100
+ SPOMe, 1 μM	93.6 ± 4.16 (6)
+ senktide, 1 μM	94.9 ± 3.40 (6)
+ atropine, 1 μM	0.0 ± 0.00* (6)

* Significant difference from 100% at $P < 0.05$.
SPOMe; substance P methyl ester.

SPOMe (1 μM) or preincubation with senktide (1 μM), but abolished by atropine (1 μM, Table 4). Desensitization with α,β-MeATP and SPOMe was carried out as illustrated in Figure 3.

Discussion

Acetylcholine output evoked by nicotine was determined by radioimmunoassay with rabbit antiserum according to the method of Kawashima *et al.* (1988). They reported that the antiserum was specific for acetylcholine and that the cross-reactivity with choline, phosphatidylcholine and phosphorylcholine was less than 0.012%. To avoid influence of the cross-reactivity with nicotine, the standard curve for acetylcholine was derived in the presence of nicotine.

In order to inhibit acetylcholinesterase activity irreversibly, the strips were incubated at 37°C for 30 min in 1 mM MSF.

Table 3 The pD_2 values of neurokinin A (NKA), Tyr^0-NKB and substance P methyl ester (SPOMe) before and after treatment with methanesulphonyl fluoride and atropine

	pD_2 value		
		After pretreatment	
	Before treatment	*MSF*	*MSF + atropine*
NKA	6.65 ± 0.11 (6)	7.21 ± 0.03* (6)	6.34 ± 0.20 (6)
Tyr^0-NKB	6.48 ± 0.13 (6)	6.50 ± 0.14 (6)	6.22 ± 0.20 (6)
SPOMe	7.43 ± 0.14 (6)	7.40 ± 0.12 (6)	7.24 ± 0.10 (6)

* Significant difference from before treatment value at $P < 0.05$.
MSF: After treatment with methanesulphonyl phluoride 1 mM for 30 min.
The pD_2 value of NKA was increased by the irreversible acetylcholinesterase inhibitor, MSF. This facilitatory effect of MSF was abolished by application of atropine (1 μM).

The pD_2 value for nicotine in the MSF-treated preparation (4.21 ± 0.06, $n = 6$) was significantly greater than in the untreated strips (3.61 ± 0.14, $n = 6$) obtained in our previous study (Hisayama *et al.*, 1988a). Nicotine increased acetylcholine release in guinea-pig derusor strips in this study. This nicotine-evoked response was markedly reduced by hexamethonium (10 μM), indicating that the effect resulted from an interaction with autonomic nicotinic receptors. On the other hand, nicotine-induced acetylcholine release was not influenced by tetrodotoxin (300 nM). It has been suggested that two mechanisms are involved in the transmitter release induced by nicotine, one dependent on sodium action potentials and the other independent (Takayanagi *et al.*, 1984; Hisayama *et al.*, 1988b). In this study it was clearly shown that nicotine evoked acetylcholine release through the latter mechanism.

We confirmed the previous finding that the final effect of the sympathetic nerve and tachykinin(s) is an increased acetylcholine release evoked by nicotine (Hisayama *et al.*, 1988a). Guanethidine and rpwwL-SP inhibited the nicotine-induced acetylcholine release to much the same degree. This inhibitory effect on the release was significantly greater than on the contraction evoked by nicotine. Application of guanethidine to block the release of mediator from the sympathetic nerve, completely abolished the inhibitory effect of rpwwL-SP on nicotine-induced acetylcholine release. Conversely, when the preparation was treated with rpwwL-SP, the inhibitory effect of guanethidine was abolished (Table 1). In other words, when the function of the sympathetic nerve was blocked, the effect of tachykinin(s) was abolished.

Nicotine-induced contraction was not antagonized by bunazosin or yohimbine although a sympathetic blocking agent, guanethidine was effective. It was known that exogenously applied noradrenaline did not cause a contraction even in the presence of noradrenaline uptake mechanisms (Hisayama *et al.*, 1988a). It seems unlikely that the effect of guanethidine was nonspecific, since chemical denervation with 6-hydroxydopamine abolished the inhibitory effect of guanethidine, and the drug did not inhibit the muscarinic receptor and purinoceptor mechanisms (Hisayama *et al.*, 1988a). The sympathomimetic effect of nicotine was non-adrenergic in nature, even if noradrenaline was released from the sympathetic nerve.

Stimulation of acetylcholine release from the cholinergic nerve by tachykinin(s) has previously been shown in guinea-pig ileum (Laufer *et al.*, 1985), spinal cord (Otsuka & Konisihi, 1975) and guinea-pig bladder (Shirakawa *et al.*, 1989). In guinea-pig ileum, NK_3 receptors on enteric neurones may play a functional role in the regulation of motility by promoting the release of acetylcholine (Laufer *et al.*, 1985; Guard & Watson, 1987). Tachykinin(s) facilitate the release of transmitter from the sympathetic nerves as a result of an interaction with NK_2 receptors (Regoli *et al.*, 1990; Tousignant *et al.*, 1987). We recently found that both NK_1 and NK_2 receptors, but not NK_3 receptors, do play an important role in the tachykinin-induced contraction in the guinea-pig urinary bladder (Shinkai & Takayanagi, 1990).

To determine the tachykinin receptor subtype involved in the cholinergic component of nicotine contraction (resistant to the desensitization of P_{2x}-purinoceptors by α,β-MeATP), the effects of two selective agonists were examined. Desensitization of NK_1 receptors by SPOMe or preincubation with the NK_3 selective agonist, senktide had no effect on the cholinergic component of nicotine contraction. NK_2 but not NK_1 or NK_3 tachykinin receptor subtypes might therefore be involved in the increased acetylcholine output evoked by nicotine. Consistent with this result, concentration-response curves to NKA were shifted to the left by treatment with the cholinesterase inhibitor, MSF and were partially inhibited by atropine, indicating that NKA directly stimulated release of acetylcholine from the nerve ending. Sensitivity to SPOMe and Tyr^0-NKB was not influenced by MSF or atropine, because the selectivity of these drugs for NK_2 receptors is relatively low (Table 4).

When applied to the neurones of isolated inferior mesenteric ganglia of guinea-pig, SP caused a membrane depolarization (Dun & Karczmer, 1979). Treatment of rats with 6-hydroxydopamine which selectively destroys principal sympathetic neurones, profoundly decreased substance P in the superior ganglion neurones (Black, 1985). Pernow (1983) suggested in his review that, in general, the level of tachykinin(s)

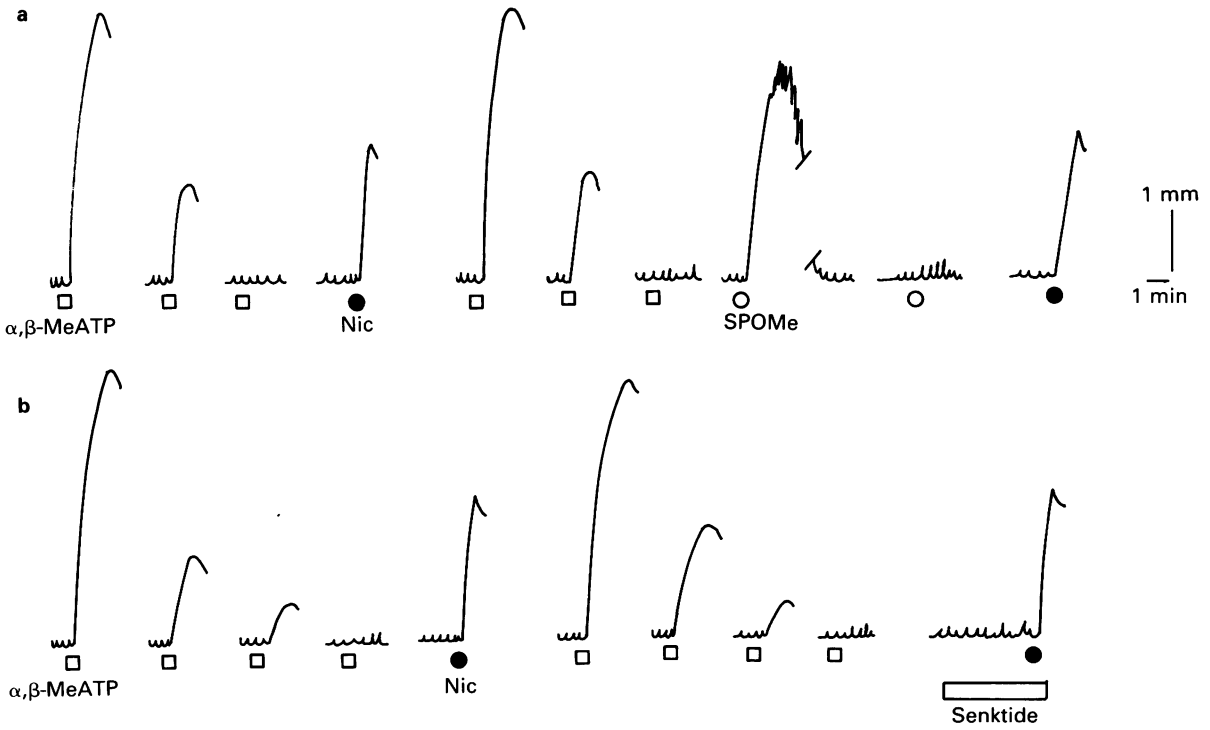

Figure 3 Typical tracings illustrating the effect of substance P methyl ester (SPOMe) desensitization (a) or preincubation with senktide (b) on the cholinergic component of the nicotine-induced contraction. α,β-Methylene ATP (α,β-MeATP) desensitization was achieved by 3 to 5 successive applications (□) at approximately 4 min intervals. Under these conditions, only the cholinergic component of nicotine contraction was observed: (●) nicotine 0.1 mM, (○) SPOMe 1 μM; open bar, senktide 1 μM.

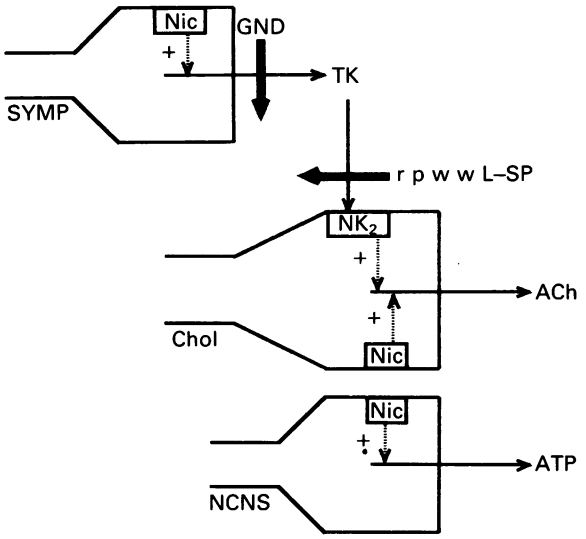

Figure 4 Postulated schemes of mechanisms of action of nicotine in guinea-pig detrusor. Nicotine may interact with the presynaptic nicotinic receptors located on (1) parasympathetic cholinergic, (2) sympathetic non-adrenergic, (3) non-cholinergic non-sympathetic purinergic nerves to induce a release of excitatory transmitters, acetylcholine and a purine nucleotide. Tachykinin(s) from capsaicin-insensitive sites (probably sympathetic nerves) causes facilitation of release of acetylcholine through activation of NK_2 tachykinin receptors. The possibility remains that a purine nucleotide is colocated with acetylcholine in parasympathetic cholinergic nerves. Further evidence for the action of nicotine in this model can be found in Hisayama *et al.* (1988a; 1989). SYMP: sympathetic nerves; Chol: parasympathetic cholinergic nerves; NCNS: non-cholinergic non-sympathetic purinergic nerves; Nic: nicotinic receptors; NK_2: NK_2 tachykinin receptors; GND: guanethidine; TK: tachykinin(s); rpwwL-SP: [D-Arg[1],D-Pro[2],D-Trp[7,9], Leu[11]]-substance P; ACh: acetylcholine.

is regulated by the sympathetic impulse activity.

Capsaicin is known to be a drug that releases and depletes tachykinins from primary sensory neurones (Hua *et al.*, 1986). We showed earlier the lack of effect of capsaicin-treatment on the nicotine-induced contraction, suggesting that sensory nerves are not involved in the action of nicotine as a site from which tachykinin(s) is released (Hisayama *et al.*, 1988a).

Nicotine-induced contraction was reduced to 30% by a muscarinic cholinoceptor antagonist, atropine. In the presence of atropine the contraction was abolished after desensitization of P_2-purinoceptors with α,β-MeATP (Table 2). Consistent with our previous data (Hisayama *et al.*, 1988a), not only acetylcholine but purine nucleotide is involved in the action of nicotine as a transmitter in guinea-pig detrusor strips treated with MSF. Since the final effect of the coordinated action of the sympathetic nerve and tachykinin(s) was only an increased acetylcholine release, the degree of inhibition of the contractile response was less than that of acetylcholine release (Table 1). Though tachykinin(s) could release acetylcholine but not purine nucleotide in our study, it is not clear whether purine nucleotide is in separate purinergic nerves, or is located with acetylcholine in cholinergic nerves.

We present postulated schemes of mechanisms of action of nicotine in guinea-pig detrusor in Figure 4. In conclusion, we have shown that nicotine stimulates acetylcholine output in a tetrodotoxin-resistant manner in guinea-pig detrusor strips, and that acetylcholine release evoked by nicotine is increased by the coordinated action of sympathetic nerves and tachykinin(s). It is further suggested that the tachykinin receptor subtype involved in the increased acetylcholine release is the NK_2 subtype in guinea-pig bladder.

This research was supported by a Grant-in-Aid (No. 02771742) for Scientific Research from the Ministry of Education Science and Culture, Japan.

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.

BLACK, I.B. (1985). *Substance P in Principal Sympathetic Neurons.* Japan Medical Research Foundation. vol. 23, pp. 23–29, ed. Tsukada, Y. Tokyo: University of Tokyo Press.

DUN, N.J. & KARCZMER, A.G. (1979). Action of substance P on sympathetic neurons. *Neuropharmacol.*, **18**, 215–218.

FALCONIERI ERSPAMER, G., ERSPAMER, V. & PICCINELLI, D. (1980). Parallel bioassay of physalaemin and kassinin, a tachykinin dodecapeptide from the skin of the African frog Kassina Senegalensis. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **311**, 61–65.

FARR, R.S. (1958). A quantitative immunological measure of the primary interaction between I*-BSA and antibody. *J. Infect. Dis.*, **103**, 239–262.

GUARD, S. & WATSON, S.P. (1987). Evidence for neurokinin-3 receptor-mediated tachykinin release in the guinea-pig ileum. *Eur. J. Pharmacol.*, **144**, 409–412.

HISAYAMA, T., SHINKAI, M., TAKAYANAGI, I. & TOYODA, T. (1988a). Mechanism of action of nicotine in isolated urinary bladder of guinea-pig. *Br. J. Pharmacol.*, **95**, 465–472.

HISAYAMA, T., SHINKAI, M., TAKAYANAGI, I., MORIMOTO, S.-I. & ISHIDA, K. (1988b). Mechanism of action of nicotine in isolated iris sphincter preparations of rabbit. *Br. J. Pharmacol.*, **95**, 459–464.

HISAYAMA, T., SHINKAI, M., TAKAYANAGI, I. & TOYODA, T. (1989). Mechanism of action of nicotine in isolated urinary bladder of guinea-pig: involvement of tachykinin(s) released by nicotine in the drug's sympathomimetic effect. *Arch. Int. Pharmacodyn. Ther.*, **301**, 277–284.

HOKFELT, 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**, Suppl. 79–89.

HUA, X.Y., SARIA, A., GAMSE, R., THEODORESSON-NORHEIM, E., BRODIN, E. & LUNDBERG, J.M. (1986). Capsaicin-induced release of multiple tachykinins (substance P, neurokinin A and eledoisin-like material) from guinea-pig spinal cord and ureter. *Neuroscience*, **19**, 313–319.

HUNTER, J.C. & MAGGIO, J.E. (1984). A pharmacological study with substance K: evidence for multiple types of tachykinin receptors. *Eur. J. Pharmacol.*, **105**, 149–153.

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 responses of the guinea-pig urinary bladder. *Eur. J. Pharmacol.*, **86**, 291–294.

KAWASHIMA, K., FUJIMOTO, K., SUZUKI, T. & OOHATA, H. (1988). Direct determination of acetylcholine release by radioimmunoassay and presence of presynaptic M_1 muscarinic receptors in guinea pig ileum. *J. Pharmacol. Exp. Ther.*, **244**, 1036–1039.

LAUFER, R., WORMSER, U., FRIEDMAN, Z.Y., GILON, C., CHOREV, M. & SELINGER, Z. (1985). Neurokinin B is a preferred agonist for a neuronal substance P receptor and its action is antagonized by enkephalin. *Proc. Natl. Acad. Sci., U.S.A.*, **82**, 7444–7448.

PERNOW, B. (1983). Substance P. *Pharmacol. Rev.*, **35**, 85–141.

OTSUKA, M. & KONISHI, S. (1975). Substance P and excitatory transmitter of primary sensory neurons. In *Cold Spring Harbor Symp. Quant. Biol.*, Vol. **40**, pp. 135–143. New York: Cold Spring Harbor Laboratory.

REGOLI, D., RHALEB, N.E., DION, S., TOUSIGNANT, C., ROUISSI, N., JUKIC, D. & DRAPEAU, G. (1990). Neurokinin A. A pharmacological study. *Pharmacol. Res.*, **22**, 1–14.

SHINKAI, M. & TAKAYANI, I. (1990). Characterization of tachykinin receptors in urinary bladder from guinea pig. *Jpn J. Pharmacol.*, **54**, 241–243.

SHIRAKAWA, J., NAKANISHI, T., TANIYAMA, K., KAMIDONO, S. & TANAKA, C. (1989). Regulation of the substance P-induced contraction via the release of acetylcholine and γ-aminobutyric acid in the guinea-pig urinary bladder. *Br. J. Pharmacol.*, **98**, 437–444.

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

TOUSIGNANT, C., DION, S., DRAPEAU, G. & REGOLI, D. (1987). Characterization of pre- and postjunctional receptors for neurokinins and kinins in the rat vas deferens. *Neuropeptides*, **9**, 333–343.

(Received October 1, 1990
Revised December 18, 1990
Accepted January 4, 1991)