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Nicotinic receptors involved in gastric noradrenaline release evoked by electrical stimulation of the splanchnic nerve in rats

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Abstract

In the present experiment, we tried to compare the functional nicotinic receptors activated by electrical stimulation of the greater splanchnic nerve (containing preganglionic sympathetic nerves) to those activated by (−)-nicotine, using the isolated rat stomach. The stomach was perfused with Krebs–Ringer solution and endogenous noradrenaline released into the perfusate was electrochemically measured using high-performance liquid chromatography. The release of noradrenaline evoked by repeated application of 30 mM (−)-nicotine rapidly declined. However, the release of noradrenaline evoked by electrical stimulation of the splanchnic nerve at 2.5 Hz was not disturbed by the appearance of tachyphylaxis for (−)-nicotine. The (−)-nicotine-induced release of noradrenaline was abolished by diltiazem, but this reagent had no effect on the electrically evoked release of noradrenaline. The electrically evoked release of noradrenaline was not influenced by atropine, but was reduced to approximately 50% by hexamethonium. This electrically evoked release of noradrenaline was not influenced by α-bungarotoxin, α-conotoxin ImI (blockers of α7 nicotinic receptors) or dihydro-β-erythroidine (a blocker of α4β2 nicotinic receptors), but was reduced to about 50% by mecamylamine (a blocker of α3β4 nicotinic receptors). The (−)-nicotine-induced release of noradrenaline has already been shown to be partially blocked by dihydro-β-erythroidine and to be abolished by mecamylamine as shown by Yokotani et al. [Eur. J. Pharmacol. 402 (2000) 223.]. These results suggest that the gastric release of noradrenaline in response to electrical stimulation of the greater splanchnic nerve is mediated by cholinergic (probably ganglionic α3β4 nicotinic receptor-mediated) and non-cholinergic mechanisms in rats. However, the functional nicotinic receptor activated by electrical stimulation of the splanchnic nerve seems to be different in character from that activated by (−)-nicotine. © 2001 Published by Elsevier Science B.V.

Keywords: Splanchnic nerve, greater; Celiac ganglion; Noradrenaline release; Stomach, rat; Nicotinic receptor; Nicotine

1. Introduction

In the past decade, a great diversity of neuronal nicotinic receptors have been detected using molecular biological techniques (Schoepfer et al., 1990; Cooper et al., 1991; Séguéla et al., 1993; Sargent, 1993; McGehee and Role, 1995). Neuronal nicotinic receptor subunits are composed of eight types of α subunit (α2–α9) and three types of β subunit (β2–β4). Three α subunits (α2, α3 and α4) form functional, heteromeric receptors in combination with β subunits (β2 and β4) and three other α subunits (α7, α8 and α9) form functional, homomeric receptors. In the peripheral autonomic ganglia such as rat superior cervical ganglia and the chick ciliary ganglia, the same complement of nicotinic genes (α3, α5, α7, β2 and β4 subunit genes) is expressed (Corriveau and Berg, 1993; Mandelzys et al., 1995). However, it is not clear which subtype of nicotinic receptors is actually involved in excitation–secretion coupling.

Previously, we reported that intravenously administered (−)-nicotine inhibits vagally stimulated gastric acid secretion by the activation of gastric sympathetic nerves in rats (Yokotani et al., 1986). Activation of gastric sympathetic nerves reduces the release of acetylcholine from vagus nerve terminals by adrenergic α-adrenoceptor-mediated mechanisms, thereby inhibiting vagally stimulated gastric acid secretion (Yokotani et al., 1984, 1993). Using the isolated, vascularly perfused rat stomach (Wang et al., 2000; Yokotani et al., 2000), we demonstrated that (−)-nicotine acts on nicotinic receptors localized on celiac ganglia, thereby evoking the release of noradrenaline from postsynaptic sympathetic nerve terminals, since this alkaloid-induced release of noradrenaline was abolished by

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tetrodotoxin, a blocker of Na^+ channels (Sershen et al., 1997).

The release of noradrenaline evoked by electrical stimulation of the greater splanchnic nerve (containing presynaptic sympathetic nerves) of the isolated rat stomach is attenuated by hexamethonium, indicating that acetylcholine released from preganglionic nerve terminals also acts on the nicotinic receptor localized on the celiac ganglia (Yokotani et al., 1992). In the present study, therefore, we attempt to compare the functional nicotinic receptor involved in gastric noradrenaline release by electrical stimulation of the greater splanchnic nerve to that activated by (−)-nicotine, using several nicotinic receptor antagonists.

2. Materials and methods

2.1. Perfusion experiments

Male Wistar rats (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) weighing about 350 g were housed for at least 2 weeks in an air-conditioned room and fasted overnight before experiments. Details of the experimental procedures were as described elsewhere (Yokotani et al., 1992). Briefly, under urethane anesthesia, the abdomen was opened with a midline incision. After ligation of the abdominal aorta just above where the celiac artery branches, a cannula was inserted into the celiac artery via an incision placed on the opposite side of the aorta, and modified Krebs–Ringer solution (pH 7.4) bubbled with a mixture of 95% O_2–5% CO_2 maintained at 37 °C was perfused at a constant flow rate of 4 ml/min. Modified Krebs–Ringer solution was composed of 117.5 mM NaCl, 4.7 mM KCl, 2.4 mM $CaCl_2$, 1.1 mM $MgCl_2$, 1.1 mM NaH_2PO_4, 25 mM $NaHCO_3$, 11.1 mM glucose, 0.1% of bovine serum albumin, 10 μM pargyline and 1 μM phentolamine. After the start of the stomach perfusion, the cannula inserted into the carotid artery was opened to exsanguinate the animal. The right side greater splanchnic nerve was cut peripherally to the bifurcation of the adrenal branch. A cannula was inserted into the lumen of the stomach via a pylorus ring to drain the contents of the stomach throughout the experiments. The esophagus, duodenum, spleen and pancreas were dissected after ligation of the vessels, and the vascularly perfused stomach connected to the intact greater splanchnic nerve was kept in a chamber prewarmed at 37 °C. In this preparation, the celiac ganglia (the gastric sympathetic ganglia) remain intact (Yokotani et al., 1992). Each 2-min effluent from the portal vein was collected in chilled tubes containing 0.5 ml of 4 N perchloric acid, 2 ng of 3,4-dihydroxybenzylamine as an internal standard, and 1 drop of 2% sodium pyrosulfite solution.

After an equilibration period of 60 min, the following experiments were done to evoke the release of noradrenaline: (1) electrical stimulation consisting of square-wave pulses (2.5 Hz, 10 mA, 2 ms duration for 1 min) was applied by bipolar platinum electrodes in the presence or absence of test reagents such as diltiazem, atropine, hexamethonium, α-bungarotoxin, α-conotoxin ImI, dihydro-β-erythroidine or mecamylamine; (2) (−)-nicotine (3×10^{-5} M) was repeatedly applied for 2 min in the perfusion medium or applied only once for 2 min in the presence or absence of diltiazem.

All experiments were conducted in compliance with the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society and the Guidelines for Animal Experiments of the Kochi Medical School.

2.2. Noradrenaline assay in the medium and the stomach

At the end of each experiment, the stomach was homogenized in 20 ml of 0.4 N perchloric acid containing 16.8 mg of disodium EDTA, 2 drops of 4% sodium pyrosulfite solution and 500 ng of 3,4-dihydroxybenzylamine as an internal standard. The homogenate was centrifuged for 10 min at 14,000 × *g* at 4 °C. The supernatant was analyzed to determine the tissue level of noradrenaline.

Catecholamines in the effluent and the supernatant of the tissue homogenate were extracted by the method of Anton and Sayre (1962) with a slight modification, and were assayed electrochemically using high-performance liquid chromatography (Yokotani et al., 1992). Specifically, to each 3 ml of acidified sample or an aliquot (0.1 ml) of supernatant was added 30 mg of activated alumina. The pH was then adjusted to 8.6 with 3 ml of 1.5 M Tris–HCl (pH 8.6) containing 0.1 M disodium EDTA, and then samples were shaken for 10 min. The supernatant was discarded and the alumina was washed three times with double-deionized water, and catecholamines were eluted with 300 μl of 4 % of acetic acid containing 0.1 mM disodium EDTA.

The high-performance liquid chromatography-electrochemical detection system consisted of a solvent delivery system (Model 880-PU; Japan Spectroscopic, Tokyo, Japan), a sample processor (Model 851-AS; Japan Spectroscopic), an ODS column (Cosmosil 5C18; Nacalai Tesque, Kyoto, Japan) and an electrochemical detector (Model CB-100; Eicom, Kyoto, Japan) equipped with a graphite electrode. The solvent system consisted of 100 mM KH_2PO_4, 0.02 mM disodium EDTA, 4.5 mM sodium octane sulfonate and 15% methanol. This assay could measure 2 pg of noradrenaline accurately.

2.3. Evaluation and statistical analyses

The amount of noradrenaline in each sample was calculated using the peak height ratio relative to that for 3,4-di-

hydroxybenzylamine, an internal standard. Fractional release of noradrenaline was calculated as percentage of the tissue content per 2 min. Basal release of noradrenaline was calculated by averaging the amount in the two subsequent samples before each electrical stimulation. The amounts of noradrenaline released above the basal level by the first and the second electrical stimulation are expressed as S_1 and S_2, and the effects of test reagents were evaluated as S_2/S_1 ratios. All values are expressed as the means ± S.E.M.

All data were analyzed by repeated-measure analysis of variance (ANOVA), followed by post hoc analysis with the Bonferroni method for comparing the control group to the groups treated with test reagents in Table 1. Student's *t*-test was used for comparing the control group treated with (−)-nicotine alone to the group treated with (−)-nicotine plus test reagent in Fig. 2B. *P* values less than 0.05 were taken to indicate significance.

2.4. Compounds

The following drugs were used: atropine sulfate, diltiazem hydrochloride, hexamethonium chloride, mecamylamine hydrochloride, (−)-nicotine hydrogen tartrate, pargyline hydrochloride, phentolamine hydrochloride (Sigma, St. Louis, MO, USA); α-bungarotoxin, dihydro-β-erythroidine hydrobromide, (Research Biochemicals International, Natick, MA, USA); α-conotoxin ImI (Peptide Institute, Osaka, Japan). All other reagents were of the highest grade available (Nacalai Tesque).

3. Results

3.1. The effect of repeated administration of (−)-nicotine on the release of noradrenaline evoked by electrical stimulation of the greater splanchnic nerve

The amount of noradrenaline remaining in the stomach was 763 ± 16 ng ($n = 63$). Spontaneous release of noradrenaline was about 0.03% of its tissue content per 2 min. In the present experiments, (−)-nicotine (3×10^{-5} M for 2 min) was applied three times after the first electrical stimulation of the greater splanchnic nerve at 2.5 Hz for 1 min and followed by the second electrical stimulation of this nerve (Fig. 1). The release of noradrenaline evoked by (−)-nicotine rapidly declined after repeated application. However, the release of noradrenaline evoked by electrical stimulation of the greater splanchnic nerve was not influenced by repeated application of (−)-nicotine. The S_2/S_1 ratio for electrical stimulation of the greater splanchnic nerve was 1.06 ± 0.11 ($n = 4$).

3.2. Effects of diltiazem on the release of noradrenaline evoked by electrical stimulation of the greater splanchnic nerve or by (−)-nicotine

The effect of diltiazem, a blocker of the L-type voltage-activated Ca^{2+} channel, was examined on the release of noradrenaline evoked by electrical stimulation of the greater splanchnic nerve at 2.5 Hz or by (−)-nicotine (3×10^{-5} M) (Fig. 2). The basal release of noradrenaline

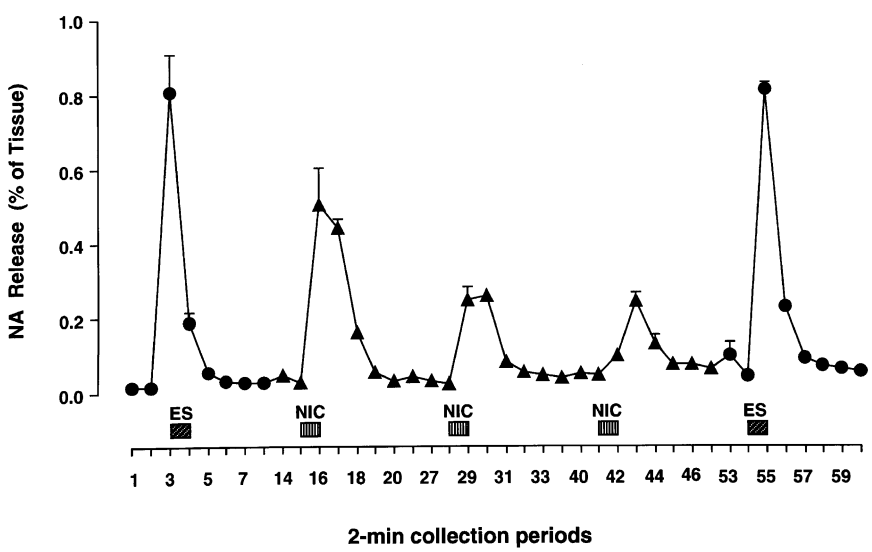

Fig. 1. Effect of repeated administration of (−)-nicotine on the release of noradrenaline (NA) evoked by electrical stimulation of the greater splanchnic nerve. (−)-Nicotine (3×10^{-5} M, for 2 min) was applied three times after the first electrical stimulation and was followed by a second electrical stimulation of the splanchnic nerve. ES, electrical stimulation of the splanchnic nerve at 2.5 Hz, 10 mA, 2 ms duration for 1 min: NIC, nicotine (3×10^{-5} M, for 2 min). NA release is expressed as percentage of tissue content per 2 min. Values are means ± S.E.M. ($n = 4$).

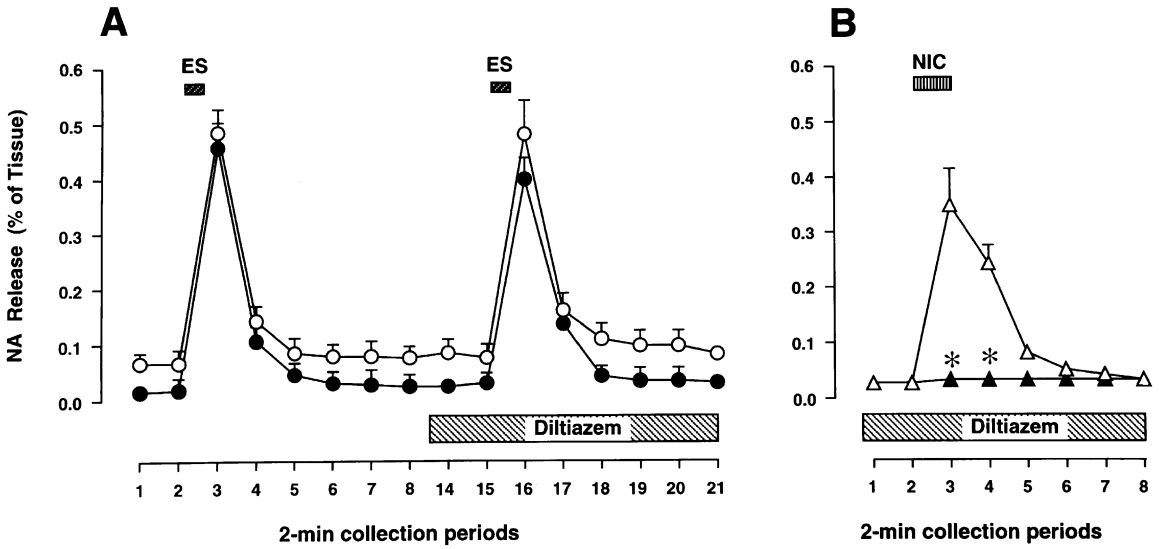

Fig. 2. Effect of diltiazem on the release of noradrenaline evoked by electrical stimulation of the greater splanchnic nerve or by (−)-nicotine. (A) Electrical stimulation (ES) of the splanchnic nerve at 2.5 Hz for 1 min: diltiazem (10^{-5} M) was added 14 min before the second electrical stimulation and was present throughout the experiment. ○, control group (n = 4); ●, diltiazem-treated group (n = 5). (B) Application of (−)-nicotine (NIC) to the perfusion medium (3×10^{-5} M for 2 min): diltiazem (10^{-5} M) was added 14 min before application of (−)-nicotine. △, control group ($n = 6$); ▲, diltiazem-treated group ($n = 5$). * Significantly different ($P < 0.05$) from the control. Other conditions were the same as those for Fig. 1.

was not affected by 10^{-5} M diltiazem. The release of noradrenaline evoked by electrical stimulation of the greater splanchnic nerve was not influenced by 10^{-5} M diltiazem (Fig. 2A); however, the (−)-nicotine-induced release of noradrenaline was abolished by this reagent (Fig. 2B). In experiments with electrical stimulation of the greater splanchnic nerve (Fig. 2A), the S_2/S_1 ratio was 1.08 ± 0.09 for the control group ($n = 4$) and 0.87 ± 0.09 for the diltiazem-treated group ($n = 5$), respectively. These values were not significantly different.

3.3. Effect of atropine, hexamethonium, α-bungarotoxin, α-conotoxin ImI, dihydro-β-erythroidine and mecamylamine on the release of noradrenaline evoked by electrical stimulation of the greater splanchnic nerve

The effects of atropine and several kinds of nicotinic receptor antagonist were examined on the release of noradrenaline evoked by electrical stimulation of the greater splanchnic nerve at 2.5 Hz (Table 1). Atropine and nicotinic receptor antagonists (hexamethonium, α-bungarotoxin, α-conotoxin ImI, dihydro-β-erythroidine and mecamylamine) had no effect on the basal release of noradrenaline.

Atropine (10^{-6} and 10^{-5} M) had no effect on the electrically evoked release of noradrenaline. Hexamethonium (10^{-4} and 5×10^{-4} M) significantly reduced this evoked response and the maximal inhibitory effect was observed at 10^{-4} M (the S_2/S_1 ratio was 0.54 ± 0.07, $n = 4$). This values was significantly different from that of control (1.06 ± 0.08, $n = 5$). However, there was no further inhibition by atropine (10^{-5} M) in addition to hexamethonium (data not shown).

The electrically evoked release of noradrenaline was not influenced by α-bungarotoxin (3×10^{-7} M), α-conotoxin ImI (10^{-6} M), or dihydro-beta-erythroidine (10^{-6} and 10^{-4} M). Mecamylamine effectively attenuated this electrically evoked response in a concentration-dependent manner (10^{-6} to 10^{-4} M). The S_2/S_1 ratio was 0.57 ± 0.05 ($n = 4$) at 10^{-4} M mecamylamine and this value was

Table 1
Effects of atropine, hexamethonium, α-bungarotoxin, α-conotoxin ImI, dihydro-β-erythroidine and mecamylamine on the release of noradrenaline evoked by electrical stimulation of the greater splanchnic nerve

Antagonist	Concentration (μM)	Number	S_2/S_1 ratio[a]
Control	–	5	1.06 ± 0.08
Atropine	1	3	1.05 ± 0.02
	10	5	1.00 ± 0.06
Hexamethonium	100	4	0.54 ± 0.07[b]
	500	4	0.55 ± 0.03[b]
α-Bungarotoxin	0.3	4	0.91 ± 0.02
α-Conotoxin ImI	1	6	0.92 ± 0.03
Dihydro-β-erythroidine	1	4	1.14 ± 0.12
	10	5	1.01 ± 0.12
	100	5	0.95 ± 0.15
Mecamylamine	1	4	0.89 ± 0.04
	10	6	0.78 ± 0.03
	100	4	0.57 ± 0.05[b]

The greater splanchnic nerve of the isolated rat stomach was electrically stimulated twice at 2.5 Hz for 1 min. Treatments with several kinds of antagonist were started 14 min before the second electrical stimulation and continued throughout the experiments. Other conditions are the same as those for Figs. 1 and 2.

[a] Effects of these reagents are expressed as S_2/S_1 ratio. All values are expressed as means ± S.E.M.

[b] Significantly different ($P < 0.05$) from the control.

significantly different from that of control (1.06 ± 0.08, $n = 5$). This inhibitory effect of mecamylamine was almost the same as that of 10^{-4} M hexamethonium.

4. Discussion

In the present experiment, the gastric release of noradrenaline evoked by (−)-nicotine declined rapidly after repeated (−)-nicotine application. However, the release of noradrenaline evoked by electrical stimulation of the greater splanchnic nerve (containing preganglionic sympathetic nerve) was not influenced by (−)-nicotine-induced desensitization. (−)-Nicotine-induced desensitization has also been demonstrated for dopamine release from rat synaptosomes (Rowell and Hillebrand, 1994). Recently, the rate of desensitization of nicotinic receptors has been shown to vary depending on the receptor subunit composition (Cachelin and Jaggi, 1991; Gross et al., 1991). This evidence suggests a difference in character between the nicotinic receptor activated by (−)-nicotine and that activated by electrical stimulation of the greater splanchnic nerve to elicit the release of noradrenaline from the rat stomach.

(−)-Nicotine-induced release of noradrenaline from the rat stomach was abolished by diltiazem (a blocker of L-type voltage-activated Ca^{2+} channels), but this reagent had no effect on the release of noradrenaline evoked by electrical stimulation of the greater splanchnic nerve (containing the presynaptic sympathetic nerve). L-Type voltage-activated Ca^{2+} channels seem to be localized on the celiac ganglia, since diltiazem had no effect on the release of noradrenaline evoked by electrical stimulation of the periarterial nerve around the left gastric artery (the postsynaptic sympathetic nerve) of the isolated rat stomach (Yokotani et al., 1998; Wang et al., 2000). Several types of voltage-activated Ca^{2+} channels are localized on sympathetic ganglia (Gonzalez-Burgos et al., 1995); however, it is not clear which type of voltage-activated Ca^{2+} channel is actually involved in excitation–secretion coupling (Carrier and Ikeda, 1992). The present results clearly demonstrate that (−)-nicotine acts on ganglionic nicotinic receptors, thereby activating ganglionic L-type voltage-activated Ca^{2+} channels to elicit the release of noradrenaline from the stomach. Electrical stimulation of the greater splanchnic nerve elicits the release of noradrenaline from the stomach without activation of L-type voltage-activated Ca^{2+} channels. These results also suggest a difference in character between nicotinic receptors activated by (−)-nicotine and those activated by electrical stimulation of the greater splanchnic nerve to elicit noradrenaline release from the rat stomach.

Next, we examined the mechanism involved in the release of noradrenaline in response to electrical stimulation of the greater splanchnic nerve. Activation of preganglionic nerve elicits several responses in ganglia (Taylor and Brown, 1998). Acetylcholine released from the preganglionic nerve terminal acts on the nicotinic receptor to generate the fast excitatory postsynaptic potential (EPSP). This is followed by several events, which amplify or suppress this signal. These include the slow EPSP; the late, slow EPSP; and an inhibitory postsynaptic potential (IPSP). The slow EPSP is generated by acetylcholine-induced activation of muscarinic receptors (Cassell and McLachlan, 1987). The late, slow EPSP is mediated by peptides found in ganglia (Vanner et al., 1993). In the present experiments, the release of noradrenaline evoked by electrical stimulation of the greater splanchnic nerve was not influenced by atropine, but was effectively attenuated by hexamethonium (approximately 50% inhibition). This suggests that the release of noradrenaline evoked by electrical stimulation of the greater splanchnic nerve is mediated by a cholinergic (nicotinic receptor-mediated) mechanism and a non-cholinergic mechanism. The release of catecholamines from rat adrenal medulla has also been shown to be stimulated by cholinergic and peptidergic transmitters released from the splanchnic nerve (Wakade, 1998).

We have already characterized the (−)-nicotine-activated nicotinic receptor localized on the celiac ganglia using several kinds of nicotinic receptor antagonist (Yokotani et al., 2000). The (−)-nicotine-induced release of noradrenaline was not influenced by α-bungarotoxin or α-conotoxin ImI (antagonists of the α7 subunit-containing nicotinic receptor) (Pereira et al., 1996), was partially blocked by dihydro-β-erythroidine (a relatively selective antagonist of the α4β2 subunit-containing nicotinic receptor) and was abolished by mecamylamine (a relatively selective antagonist of the α3β4 subunit-containing nicotinic receptor). In the present experiments, the electrically evoked release of noradrenaline was not influenced by α-bungarotoxin, α-conotoxin ImI or dihydro-β-erythroidine, but was reduced to approximately 50% by mecamylamine. This mecamylamine-induced inhibition was almost the same as that induced by hexamethonium (approximately 50% inhibition), indicating that mecamylamine abolishes the ganglionic nicotinic receptor-mediated release of noradrenaline elicited by electrical stimulation of the greater splanchnic nerve. Mecamylamine has been shown to be more effective than dihydro-β-erythroidine at α3β4 subunit-containing nicotinic receptors and dihydro-β-erythroidine is most effective at α3β2 subunit-containing nicotinic receptors (Luetje and Patrick, 1990; Alkondon and Albuquerque, 1993; Cachelin and Rust, 1995). Based on these findings, it seems likely that (−)-nicotine activates α3β2 and/or α3β4 subunit-containing nicotinic receptors and that acetylcholine released from preganglionic sympathetic nerve terminals activates α3β4 subunit-containing nicotinic receptors localized on celiac ganglia.

The involvement of voltage-activated Ca^{2+} channels has also been shown in nicotine-induced [^{3}H]-dopamine release from rat striatal synaptosomes (Prince et al., 1996; Soliakov and Wonnacott, 1996). In the apical dendrites of

pyramidal cells of the rat neocortex, a subthreshold excitatory postsynaptic potential, mediated by the activation of glutamate receptors, causes an increase in dendritic $[Ca^{2+}]_i$ by activation of voltage-activated Ca^{2+} channels (Markram and Sakmann, 1994). In the present study, therefore, it seems likely that the binding of (−)-nicotine to nicotinic receptors (probably localized on the extrasynaptic surface of the ganglia) initiates an excitatory postsynaptic potential, thereby activating ganglionic L-type voltage-activated Ca^{2+} channels. Na^+ and Ca^{2+} entries into the sympathetic ganglia through nicotinic receptors and L-type Ca^{2+} channels evoke an action potential sufficient to initiate noradrenaline release. Acetylcholine released from preganglionic nerve terminals seems to activate nicotinic receptors (probably localized on the intrasynaptic active zone of the ganglionic postsynaptic surface), thereby evoking an action potential sufficient to initiate noradrenaline release without activation of L-type voltage-activated Ca^{2+} channels.

In conclusion, functional nicotinic receptors activated by electrical stimulation of the greater splanchnic nerve (preganglionic sympathetic nerve) seem to be different in character from those activated by (−)-nicotine to elicit the release of noradrenaline from the rat stomach.

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References

Alkondon, M., Albuquerque, E.X., 1993. Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons: I. Pharmacological and functional evidence for distinct structural subtypes. J. Pharmacol. Exp. Ther. 265, 1455–1473.

Anton, A.H., Sayre, D.F., 1962. A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. J. Pharmacol. Exp. Ther. 138, 360–375.

Cachelin, A.B., Jaggi, R., 1991. Beta subunits determine the time course of desensitization in rat alpha 3 neuronal nicotinic acetylcholine receptors. Pfluegers Arch. Eur. J. Physiol. 419, 579–582.

Cachelin, A.B., Rust, G., 1995. β-subunits co-determine the sensitivity of rat neuronal nicotinic receptors to antagonists. Pfluegers Arch. Eur. J. Physiol. 429, 449–451.

Carrier, G.O., Ikeda, S.R., 1992. TTX-sensitive Na^+ channels and Ca^{2+} channels of the L- and N-type underlie the inward current in acutely dispersed coeliac-mesenteric ganglia neurons of adult rats. Pfluegers Arch. Eur. J. Physiol. 421, 7–16.

Cassell, J.F., McLachlan, E.M., 1987. Muscarinic agonists block five different potassium conductances in guinea-pig sympathetic neurones. Br. J. Pharmacol. 91, 259–261.

Cooper, E., Couturier, S., Ballivet, M., 1991. Pentameric structure and subunit stoichiometry of a neuronal nicotinic acetylcholine receptor. Nature 350, 235–238.

Corriveau, R.A., Berg, D.K., 1993. Coexpression of multiple acetylcholine receptor genes in neurons: quantification of transcripts during development. J. Neurosci. 13, 2662–2671.

Gonzalez-Burgos, G.R., Biali, F.I., Cherksey, B.D., Sugimori, M., Llinas, R.R., Uchitel, O.D., 1995. Different calcium channels mediate transmitter release evoked by transient or sustained depolarization at mammalian sympathetic ganglia. Neuroscience 64, 117–123.

Gross, A., Ballivet, M., Rungger, D., Bertrand, D., 1991. Neuronal nicotinic acetylcholine receptors expressed in Xenopus oocytes: role of the alpha subunit in agonist sensitivity and desensitization. Pfluegers Arch. Eur. J. Physiol. 419, 545–551.

Luetje, C.W., Patrick, J., 1991. Both alpha- and beta-subunits contribute to the agonist sensitivity of neuronal nicotinic acetylcholine receptors. J. Neurosci. 11, 837–845.

Mandelzys, A., De Koninck, P., Cooper, E., 1995. Agonist and toxin sensitivities of ACh-evoked currents on neurons expressing multiple nicotinic ACh receptor subunits. J. Neurophysiol. 74, 1212–1221.

Markram, H., Sakmann, B., 1994. Calcium transients in dendrites of neocortical neurons evoked by single subthreshold excitatory postsynaptic potentials via low-voltage-activated calcium channels. Proc. Natl. Acad. Sci. U. S. A. 91, 5207–5211.

McGehee, D.S., Role, L.W., 1995. Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. Annu. Rev. Physiol. 57, 521–546.

Pereira, E.F., Alkondon, M., McIntosh, J.M., Albuquerque, E.X., 1996. Alpha-conotoxin-ImI: a competitive antagonist at alpha-bungarotoxin-sensitive neuronal nicotinic receptors in hippocampal neurons. J. Pharmacol. Exp. Ther. 278, 1472–1483.

Prince, R.J., Fernandes, K.G., Gregory, J.C., Martyn, I.D., Lippiello, P.M., 1996. Modulation of nicotine-evoked [^{3}H]dopamine release from rat striatal synaptosomes by voltage-sensitive calcium channel ligands. Biochem. Pharmacol. 52, 613–618.

Rowell, P.P., Hillebrand, J.A., 1994. Characterization of nicotine-induced desensitization of evoked dopamine release from rat striatal synaptosomes. J. Neurochem. 63, 561–569.

Sargent, P.B., 1993. The diversity of neuronal nicotinic acetylcholine receptors. Annu. Rev. Neurosci. 16, 404–443.

Schoepfer, R., Conroy, W.G., Whiting, P., Gore, M., Lindstrom, J., 1990. Brain alpha-bungarotoxin binding protein cDNAs and MAbs reveal subtypes of this branch of the ligand-gated ion channel gene superfamily. Neuron 5, 35–48.

Séguéla, P., Wadiche, J., Dineley-Miller, K., Dani, J.A., Patrick, J.W., 1993. Molecular cloning, functional properties, and distribution of rat brain alpha 7: a nicotinic cation channel highly permeable to calcium. J. Neurosci. 13, 596–604.

Sershen, H., Balla, A., Lajtha, A., Vizi, E.S., 1997. Characterization of nicotinic receptors involved in the release of noradrenaline from the hippocampus. Neuroscience 77, 121–130.

Soliakov, L., Wonnacott, S., 1996. Voltage-sensitive Ca^{2+} channels involved in nicotinic receptor-mediated [^{3}H]dopamine release from rat striatal synaptosomes. J. Neurochem. 67, 163–170.

Taylor, P., Brown, J.H., 1998. Acetylcholine. In: Siegel, G.J., Agranoff, B.W., Wayne-Albers, R., Fisher, S.K., Uhler, M.D. (Eds.), Basic Neurochemistry. Lippincott-Raven, Philadelphia, pp. 213–242.

Vanner, S., Evans, R.J., Matsumoto, S.G., Surprenant, A., 1993. Potassium currents and their modulation by muscarine and substance P in neuronal cultures from adult guinea pig celiac ganglia. J. Neurophysiol. 69, 1632–1644.

Wakade, A.R., 1998. Multiple transmitter control of catecholamine secretion in rat adrenal medulla. Adv. Pharmacol. 42, 595–598.

Wang, M., Murakami, Y., Okada, S., Yokotani, K., 2000. Nicotine-induced noradrenaline release from the isolated rat stomach by activation of L- and N-type calcium channels. Jpn. J. Pharmacol. 83, 102–106.

Yokotani, K., Muramatsu, I., Fujiwara, M., 1984. Alpha-1 and alpha-2 type adrenoceptors involved in the inhibitory effect of splanchnic nerves on parasympathetically stimulated gastric acid secretion in rats. J. Pharmacol. Exp. Ther. 229, 305–310.

Yokotani, K., Okuma, Y., Osumi, Y., 1986. Sympatho-adrenal system involved in the inhibitory effects of nicotine on the vagally stimulated gastric acid output and mucosal blood flow in rats. Eur. J. Pharmacol. 129, 253–260.

Yokotani, K., Okuma, Y., Osumi, Y., 1992. Release of endogenous noradrenaline from the vascularly perfused rat stomach in vitro; modulation by pre- and postsynaptic adrenoceptors. J. Pharmacol. Exp. Ther. 260, 728–733.

Yokotani, K., Okuma, Y., Nakamura, K., Osumi, Y., 1993. Release of endogenous acetylcholine from a vascularly perfused rat stomach in vitro; inhibition by M3 muscarinic autoreceptors and alpha-2 adrenoceptors. J. Pharmacol. Exp. Ther. 266, 1190–1195.

Yokotani, K., Okuma, Y., Osumi, Y., 1998. Involvement of N-type voltage-activated Ca^{2+} channels in the release of endogenous noradrenaline from the isolated vascularly perfused rat stomach. Jpn. J. Pharmacol. 78, 75–77.

Yokotani, K., Wang, M., Okada, S., Murakami, Y., Hirata, M., 2000. Characterization of nicotinic acetylcholine receptor-mediated noradrenaline release from the isolated rat stomach. Eur. J. Pharmacol. 402, 223–229.