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Characterization of functional nicotinic acetylcholine receptors involved in catecholamine release from the isolated rat adrenal gland

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Abstract

We tried to characterize nicotinic acetylcholine receptors involved in the release of catecholamines from the rat adrenal gland. The isolated adrenal gland was retrogradely perfused via the adrenal vein with Krebs–Ringer solution at a flow rate of 0.5 ml/min. Endogenous catecholamines, adrenaline and noradrenaline, released into the perfusate were electrochemically measured using high-performance liquid chromatography. (−)-Nicotine (3×10^{-6}–3×10^{-5} M) evoked the release of catecholamines (adrenaline≫noradrenaline) in a concentration-dependent manner. The (−)-nicotine (10^{-5} M)-induced release of catecholamines was effectively attenuated by mecamylamine (10^{-7} and 10^{-6} M) (a relatively selective antagonist of α3β4 nicotinic receptors), but not influenced by α-bungarotoxin (3×10^{-7} M) (an antagonist of α7 nicotinic receptors) and dihydro-β-erythroidine (10^{-5} M) (a relatively selective antagonist of α4β2 nicotinic receptors). (±)-Epibatidine (3×10^{-7} and 10^{-6} M) (a non-selective nicotinic receptor agonist), (−)-cytisine (10^{-5} and 10^{-4} M) (an agonist of β4 nicotinic receptors) and (±)-2-(3-pyridinyl)-1-azabicyclo(2.2.2)octane (RJR-2429) (10^{-5} M) (a putative agonist of α3β4 nicotinic receptors) effectively evoked the release of catecholamines (adrenaline≫noradrenaline), while (*E*)-*N*-methyl-4-(3-pyridinyl)-3-butene-1-amine (RJR-2403) (up to 10^{-4} M) (a selective agonist of α4β2 nicotinic receptors) had no effect. The efficacies of these agonists are as follows: (±) epibatidine≫RJR-2429>(−)-cytisine>(−)-nicotine≫RJR-2403. These results suggest that α3β4 nicotinic receptors are involved in the release of catecholamines from the rat adrenal gland.

Keywords: Nicotine; Adrenaline; Noradrenaline; Adrenal gland; (Rat); Nicotinic receptor

1. Introduction

Neuronal nicotinic acetylcholine receptors are composed of multiple subunits: eight types of α subunit (α2–α9) and three types of β subunit (β2–β4) (Sargent, 1993; McGehee, 1999). Three α subunits (α2, α3 and α4) have been shown to form heteromeric receptors in combination with a β subunit (β2 or β4) (Boulter et al., 1987; Wada et al., 1988; Cooper et al., 1991), whereas three other α subunits (α7, α8 and α9) form homomeric receptors in a recombinant expression system (Schoepfer et al., 1990; Séguéla et al., 1993). The relative efficacy and potency of available nicotinic receptor agonists and antagonists have also been defined by these recombinant expression studies (Brioni et al., 1997; Holladay et al., 1997; Lloyd and Williams, 2000). Therefore, the current study attempts to identify pharmacologically the subunits of functional nicotinic receptors involved in physiological and pathophysiological responses.

Chromaffin cells of the adrenal medulla are cholinergically innervated by the adrenal branch of the splanchnic nerves from the sympathetic nervous system. Acetylcholine released from these preganglionic sympathetic nerves activates neuronal nicotinic receptors located on chromaffin cells and the subsequent depolarization triggers catecholamine secretion. Although the expression of α3, α5, α7, β2 and β4 subunits in PC-12 cells (Rogers et al., 1992) and α3, α5 and β4 subunits in bovine adrenal chromaffin cells (Campos-Caro et al., 1997) has been reported, a limited information is available about the subunits of functional nicotinic receptors involved in catecholamine secretion. Nicotinic receptors that bind to α-bungarotoxin (a blocker of nicotinic receptors containing α7 subunit) have been shown to be not involved in adrenal secretion (Afar et al., 1994). Another nicotinic receptors interacting with the monoclonal antibody 35, which is reacting with nicotinic receptors containing α3, α5, β2 and β4 subunits (Vernallis

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et al., 1993), have been shown to be involved in adrenal secretion (Gu et al., 1996; Wenger et al., 1997). Recently, the nicotinic receptors associated with catecholamine secretion from bovine adrenal chromaffin cells have been shown to be containing at least α3β4 or α3β4α5 subunits (Tachikawa et al., 2001).

Adrenal chromaffin cells arise developmentally from the neural crest together with other cells such as postganglionic sympathetic neurons (Anderson, 1993). Recently we reported that the α3β4 subunits-containing nicotinic receptors are involved in the (−)-nicotine-induced release of noradrenaline from postganglionic sympathetic neurons of the rat stomach (Wang et al., 2000; Yokotani et al., 2000, 2001). In the present study, therefore, we attempted to characterize the functional nicotinic receptors involved in the release of catecholamines from the rat adrenal gland and compared these receptors with those involved in the gastric noradrenaline release, using several kinds of agonists and antagonists of nicotinic receptors.

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 the experiments were performed. Retrograde perfusion of the isolated adrenal gland was carried out by the method of Wakade (1981) with some modifications. Briefly, under urethane anesthesia, the abdomen was opened with a midline incision. After ligations of the left renal artery and vein at the sites where they connected with the aorta or the inferior vena cava, a cannula was inserted into the adrenal vein via an incision placed on the left renal vein 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 0.5 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 and 10 μM pargyline. A small slit was made into the adrenal cortex just opposite the entrance of the adrenal vein. The renal artery and vein, all the branches of the adrenal vein and the adrenal branch of the splanchnic nerve were dissected after ligation, and the vascularly perfused adrenal gland was kept in a chamber prewarmed at 37 °C. Each 3 min effluent escaped from a slit made into the adrenal cortex 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 one drop of 2% sodium pyrosulfite solution.

After an equilibration period of 60 min, the following experiments were done: (1) (−)-nicotine was applied for 3 min in the perfusion medium in the presence or absence of nicotinic receptor antagonists such as α-bungarotoxin, dihydro-β-erythroidine or mecamylamine; (2) nicotinic receptor agonists such as (−)-nicotine, (±)-epibatidine, (−)-cytisine, (±)-2-(3-pyridinyl)-1-azabicyclo(2.2.2)octane (RJR-2429) and (*E*)-*N*-methyl-4-(3-pyridinyl)-3-butene-1-amine (RJR-2403) were applied for 3 min in the perfusion medium. Each nicotinic receptor agonist was applied once to each preparation to avoid the appearance of tachyphylaxis after repeated administration.

All experiments were conducted in compliance with Guidelines for Animal Experiments of the Kochi Medical School.

2.2. Catecholamine assay in the medium and the adrenal gland

Catecholamines in the perfusate 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 0.1 ml of acidified sample 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 adrenaline and noradrenaline accurately.

2.3. Evaluation and statistical analysis

The amount of catecholamines (adrenaline and noradrenaline) in each sample was calculated using the peak height ratio relative to that for 3,4-dihydroxybenzylamine, an internal standard. Spontaneous and evoked release of adrenaline and noradrenaline is expressed as nanograms per 3-min collection period. All values are expressed as the means ± S.E.M.

Data were analyzed by a repeated-measure analysis of variance (ANOVA), followed by post-hoc analysis with the Bonferroni method for comparing the group treated with (−)-nicotine alone to the groups treated with (−)-nicotine plus nicotinic receptor antagonists (Fig. 1) or treated with nicotinic receptor agonists (Fig. 2). When only two means were compared, an unpaired Student's *t*-test was used

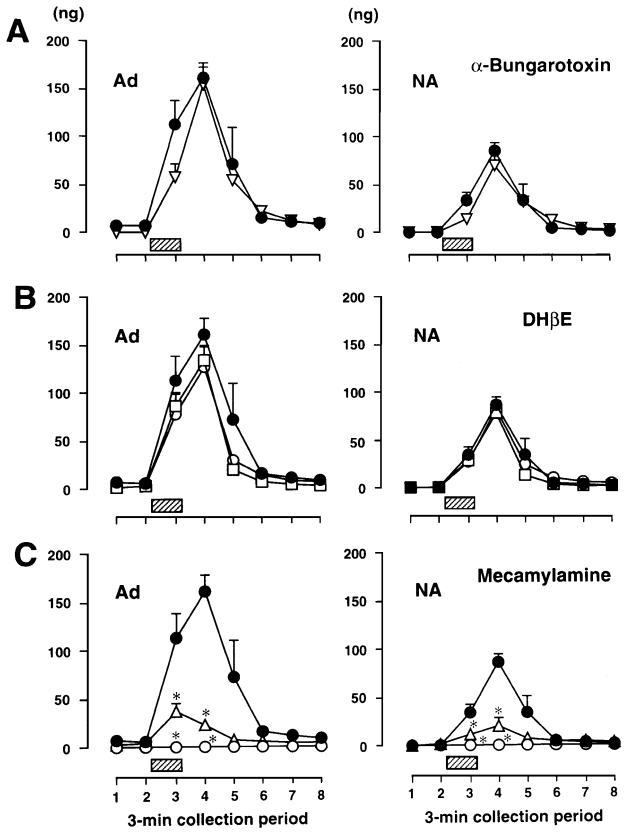

Fig. 1. Effects of α-bungarotoxin, dihydro-β-erythroidine (DHβE) and mecamylamine on the (−)-nicotine-induced release of catecholamines from the rat adrenal gland. These antagonists of nicotinic receptors were administered 20 min before the start of the experiment and continued throughout the experiment. (−)-Nicotine (10^{-5} M) was applied in the perfusion medium for 3 min (shown as shaded bar). Ad, adrenaline; NA, noradrenaline. The release of Ad and NA is expressed as nanograms per 3 min. Values are the means ± S.E.M. * Significantly different ($P<0.05$) from the control treated with (−)-nicotine alone. (A) ●, (−)-nicotine alone ($n=5$); ▽, 3×10^{-7} M α-bungarotoxin plus (−)-nicotine ($n=5$). (B) ●, (−)-nicotine alone (cited from A); ○, 10^{-6} M DHβE plus (−)-nicotine ($n=4$); □, 10^{-5} M DHβE plus (−)-nicotine ($n=5$). (C) ●, (−)-nicotine alone (cited from A); △, 10^{-7} M mecamylamine plus (−)-nicotine ($n=4$); ○, 10^{-6} M mecamylamine plus (−)-nicotine ($n=6$).

(Figs. 1 and 2). *P* values less than 0.05 were taken to indicate significance.

2.4. Compounds

The following drugs were used: (−)-cytisine, 3,4-dihydroxybenzylamine hydrobromide, hexamethonium chloride, mecamylamine hydrochloride, (−)-nicotine hydrogen tartrate, pargyline hydrochloride (Sigma, St. Louis, MO, USA); α-bungarotoxin, dihydro-β-erythroidine hydrobromide, (±)-epibatidine dihydrochloride, RJR-2429 (Research Biochemicals International, Natick, MA, USA); RJR-2403 (Tocris Cookson, Ballwin, MO, USA). All other reagents were of the highest grade available (Nacalai Tesque, Kyoto, Japan).

3. Results

3.1. Effects of nicotinic receptor antagonists on the (−)-nicotine-induced release of catecholamines from the isolated adrenal gland

The amount of adrenaline and noradrenaline remaining in the adrenal gland was 19.8 ± 0.9 and 4.5 ± 0.3 μg per gland, respectively ($n=43$). The spontaneous release of adrenaline and noradrenaline from the isolated, vascularly perfused adrenal gland was 4.6 ± 1.2 and 1.0 ± 0.3 ng per 3 min

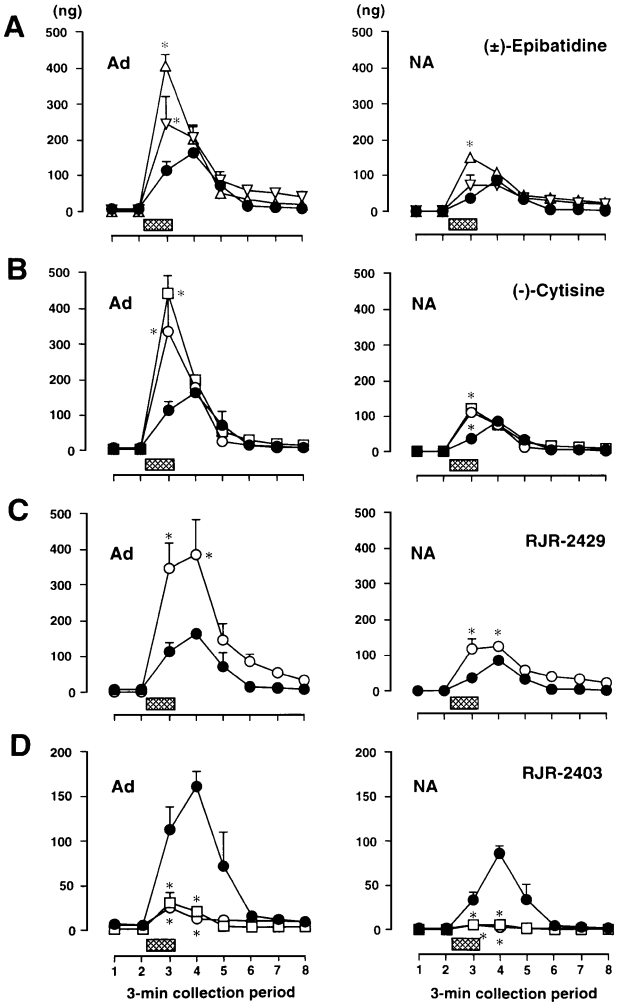

Fig. 2. Effects of (±)-epibatidine, (−)-cytisine, RJR-2429 and RJR-2403 on the release of catecholamines from the rat adrenal gland. These effects were compared with (−)-nicotine (10^{-5} M). These agonists of nicotinic receptors were applied in the perfusion medium for 3 min (shown as hatched bar). * Significantly different ($P<0.05$) from the group treated with 10^{-5} M (−)-nicotine. Other conditions were the same as those for Fig. 1. (A) ●, 10^{-5} M (−)-nicotine alone (cited from Fig. 1A); ▽, 3×10^{-7} M (±)-epibatidine ($n=4$); △, 10^{-6} M (±)-epibatidine ($n=4$). (B) ●, 10^{-5} M (−)-nicotine alone (cited from Fig. 1A); ○, 10^{-5} M (−)-cytisine ($n=4$); □, 10^{-4} M (−)-cytisine ($n=5$). (C) ●, 10^{-5} M (−)-nicotine alone (cited from Fig. 1A); ○, 10^{-5} M RJR-2429 ($n=5$). (D) ●, 10^{-5} M (−)-nicotine alone (cited from Fig. 1A); ○, 10^{-5} M RJR-2403 ($n=4$); □, 10^{-4} M RJR-2403 ($n=4$).

($n=43$). (−)-Nicotine (3×10^{-6}–3×10^{-5} M) evoked the release of both catecholamines (adrenaline≫noradrenaline) in a concentration-dependent manner. After application of this alkaloid, the evoked levels of both catecholamines quickly declined to their basal levels (Fig. 1A). The (−)-nicotine (10^{-5} M)-induced release of catecholamines was abolished by hexamethonium (3×10^{-5} M). In the following experiments, 10^{-5} M (−)-nicotine was always used.

The effects of nicotinic receptor antagonists, α-bungarotoxin, dihydro-β-erythroidine and mecamylamine, on the (−)-nicotine-induced release of catecholamines were examined (Fig. 1). The basal release of both catecholamines was not influenced by these nicotinic receptor antagonists (Fig. 1A–C). The release of adrenaline and noradrenaline evoked by (−)-nicotine (10^{-5} M) was not influenced by α-bungarotoxin (3×10^{-7} M) and dihydro-β-erythroidine (10^{-6} and 10^{-5} M) (Fig. 1A and B). On the other hand, mecamylamine (10^{-7} and 10^{-6} M) effectively reduced this alkaloid-induced release of both catecholamines in a concentration-dependent manner (Fig. 1C).

3.2. Effects of nicotinic receptor agonists on the release of catecholamines from the isolated adrenal gland

The effects of several kinds of nicotinic receptor agonists such as (±)-epibatidine, (−)-cytisine, RJR-2429 and RJR-2403 were compared with that of (−)-nicotine (10^{-5} M) on the release of catecholamines from the adrenal gland (Fig. 2).

(±)-Epibatidine (3×10^{-7} and 10^{-6} M), (−)-cytisine (10^{-5} and 10^{-4} M) and RJR-2429 (10^{-5} M) markedly increased the release of both catecholamines from the adrenal gland (adrenaline≫noradrenaline) (Fig. 2A–C). After application of these reagents, the evoked release of both catecholamines quickly declined to their basal levels. On the other hand, RJR-2403 (10^{-5} and 10^{-4} M) had no effect on the release of both catecholamines (Fig. 2D). The efficacies of these agonists on the release of catecholamines were as follows: (±)-epibatidine≫RJR-2429>(−)-cytisine>(−)-nicotine≫RJR-2403.

4. Discussion

In the first experiments, we examined the effects of nicotinic receptor antagonists on the (−)-nicotine-induced release of catecholamines from the rat adrenal gland. The (−)-nicotine-induced release of catecholamines (adrenaline≫noradrenaline) was abolished by hexamethonium (a nonselective antagonist of nicotinic receptors), but not influenced by α-bungarotoxin [an antagonist of the nicotinic receptors containing α7 subunit (Couturier et al., 1990)], suggesting that the nicotinic receptors containing α7 subunit are not involved in the (−)-nicotine-induced release of catecholamines from the adrenal gland. These results are also observed in the autonomic ganglia, in which the nicotinic receptors containing α7 subunit are prominent, but not involved in ganglionic transmission (Chiappinelli et al., 1981).

In the present experiments, the (−)-nicotine-induced release of catecholamines was effectively attenuated by mecamylamine, but not influenced by dihydro-β-erythroidine. Mecamylamine has been shown to be more effective than dihydro-β-erythroidine at the nicotinic receptors containing α3β4 subunits and dihydro-β-erythroidine is more effective at the nicotinic receptors containing α4β2 subunits than those containing α3β2 subunits in oocyte transfection studies (Luetje and Patrick, 1991; Alkondon and Albuquerque, 1993; Cachelin and Rust, 1995; Xiao et al., 1998). Based on these evidence, our observations favor the hypothesis that α3β4 subunits-containing nicotinic receptors are involved in the (−)-nicotine-induced release of catecholamines from the rat adrenal gland, as shown by Tachikawa et al. (2001).

In the next experiments, we examined the effects of nicotinic receptor agonists on the release of catecholamines from the rat adrenal gland. (±)-Epibatidine, (−)-cytisine and RJR-2429 markedly increased the release of both catecholamines from the adrenal gland (adrenaline≫noradrenaline); however, RJR-2403 had no effect. The efficacies of these agonists were as follows: (±)-epibatidine≫RJR-2429>(−)-cytisine>(−)-nicotine≫RJR-2403. (±)-Epibatidine, an alkaloid isolated from the skin of the Ecuadorian frog, *Epipedobates tricoloris* (Spande et al., 1992), is a potent but non-selective agonist of nicotinic receptors and is a full agonist at the nicotinic receptors containing α4β2, α3β2, α3β4 or α7 subunits (Fisher et al., 1994; Sacaan et al., 1996). (−)-Cytisine is a potent agonist of the nicotinic receptors containing β4 subunit but has little effect on those containing β2 subunit (Luetje and Patrick, 1991; Papke and Heinemann, 1994), and has been shown to evoke the release of catecholamines from bovine adrenal chromaffin cells with somewhat less efficacy than (−)-nicotine (Wenger et al., 1997). RJR-2429 is a putative activator of nicotinic receptors containing α3β4 subunits in PC12 cells and the efficacy of this reagent has been shown to be intermediate between (±)-epibatidine and (−)-nicotine (Bencherif et al., 1998). RJR-2403 is a marked selective agonist of nicotinic receptors containing α4β2 subunits (Bencherif et al., 1996). Based on these evidence, our present results suggest the involvement of the nicotinic receptors containing α3β4 subunits in the release of catecholamines from the rat adrenal gland.

In conclusion, the nicotinic receptors containing α3β4 subunits are probably involved in the release of catecholamines from the rat adrenal chromaffin cells, as shown in the release of noradrenaline from the rat gastric sympathetic nerves.

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References

Afar, R., Trifaro, J.M., Quik, M., 1994. Nicotine-induced intracellular calcium changes are not antagonized by alpha-bungarotoxin in adrenal medullary cells. Brain Res. 641, 127–131.

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.

Anderson, D.J., 1993. Molecular control of cell fate in the neural crest: the sympathoadrenal lineage. Annu. Rev. Neurosci. 16, 129–158.

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.

Bencherif, M., Lovette, M.E., Fowler, K.W., Arrington, S., Reeves, L., Caldwell, W.S., Lippiello, P.M., 1996. RJR-2403: a nicotinic agonist with CNS selectivity: I. In vitro characterization. J. Pharmacol. Exp. Ther. 279, 1413–1421.

Bencherif, M., Schmitt, J.D., Bhatti, B.S., Crooks, P., Caldwell, W.S., Lovette, M.E., Fowler, K., Reeves, L., Lippiello, P.M., 1998. The heterocyclic substituted pyridine derivative (±)-2-(-3-pyridinyl)-1-azabicyclo[2.2.2]octane (RJR-2429): a selective ligand at nicotinic acetylcholine receptors. J. Pharmacol. Exp. Ther. 284, 886–894.

Boulter, J., Connolly, J., Deneris, E., Goldman, D., Heinemann, S., Patrick, J., 1987. Functional expression of two neuronal nicotinic acetylcholine receptors from cDNA clones identifies a gene family. Proc. Natl. Acad. Sci. U. S. A. 84, 7763–7767.

Brioni, J.D., Decker, M.W., Sullivan, J.P., Arneric, S.P., 1997. The pharmacology of (−)-nicotine and novel cholinergic channel modulators. Adv. Pharmacol. (N. Y.) 37, 153–214.

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.

Campos-Caro, A., Smillie, F.I., Dominguez del Toro, E., Rovira, J.C., Vicente-Agullo, F., Chapuli, J., Juiz, J.M., Sala, S., Sala, F., Ballesta, J.J., Criado, M., 1997. Neuronal nicotinic acetylcholine receptors on bovine chromaffin cells: cloning, expression, and genomic organization of receptor subunits. J. Neurochem. 68, 488–497.

Chiappinelli, V.A., Cohen, J.B., Zigmond, R.E., 1981. The effects of alpha- and beta-neurotoxins from the venoms of various snakes on transmission in autonomic ganglia. Brain Res. 211, 107–126.

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

Couturier, S., Bertrand, D., Matter, J.M., Hernandez, M.C., Bertrand, S., Millar, N., Valera, S., Barkas, T., Ballivet, M., 1990. A neuronal nicotinic acetylcholine receptor subunit (alpha 7) is developmentally regulated and forms a homo-oligomeric channel blocked by alpha-BTX. Neuron 5, 847–856.

Fisher, M., Huangfu, D., Shen, T.Y., Guyenet, P.G., 1994. Epibatidine, an alkaloid from the poison frog *Epipedobates tricolor*, is a powerful ganglionic depolarizing agent. J. Pharmacol. Exp. Ther. 270, 702–707.

Gu, H., Wenger, B.W., Lopez, I., McKay, S.B., Boyd, R.T., McKay, D.B., 1996. Characterization and localization of adrenal nicotinic acetylcholine receptors: evidence that mAb35-nicotinic receptors are the principal receptors mediating adrenal catecholamine secretion. J. Neurochem. 66, 1454–1461.

Holladay, M.W., Dart, M.J., Lynch, J.K., 1997. Neuronal nicotinic acetylcholine receptors as targets for drug discovery. J. Med. Chem. 40, 4169–4194.

Lloyd, G.K., Williams, M., 2000. Neuronal nicotinic acetylcholine receptors as novel drug targets. J. Pharmacol. Exp. Ther. 292, 461–467.

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.

McGehee, D.S., 1999. Molecular diversity of neuronal nicotinic acetylcholine receptors. Ann. N. Y. Acad. Sci. 868, 565–577.

Papke, R.L., Heinemann, S.F., 1994. Partial agonist properties of cytisine on neuronal nicotinic receptors containing the beta 2 subunit. Mol. Pharmacol. 45, 142–149.

Rogers, S.W., Mandelzys, A., Deneris, E.S., Cooper, E., Heinemann, S., 1992. The expression of nicotinic acetylcholine receptors by PC12 cells treated with NGF. J. Neurosci. 12, 4611–4623.

Sacaan, A.I., Menzaghi, F., Dunlop, J.L., Correa, L.D., Whelan, K.T., Lloyd, G.K., 1996. Epibatidine: a nicotinic acetylcholine receptor agonist releases monoaminergic neurotransmitters: in vitro and in vivo evidence in rats. J. Pharmacol. Exp. Ther. 276, 509–515.

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.

Spande, T.F., Garraffo, H.M., Yeh, H.J., Pu, Q.L., Pannell, L.K., Daly, J.W., 1992. A new class of alkaloids from a dendrobatid poison frog: a structure for alkaloid 251F. J. Nat. Prod. 55, 707–722.

Tachikawa, E., Mizuma, K., Kudo, K., Kashimoto, T., Yamato, S., Ohta, S., 2001. Characterization of the functional subunit combination of nicotinic acetylcholine receptors in bovine adrenal chromaffin cells. Neurosci. Lett. 26 (312), 161–164.

Vernallis, A.B., Conroy, W.G., Berg, D.K., 1993. Neurons assemble acetylcholine receptors with as many as three kinds of subunits while maintaining subunit segregation among receptor subtypes. Neuron 10, 451–464.

Wada, K., Ballivet, M., Boulter, J., Connolly, J., Wada, E., Deneris, E.S., Swanson, L.W., Heinemann, S., Patrick, J., 1988. Functional expression of a new pharmacological subtype of brain nicotinic acetylcholine receptor. Science 240, 330–334.

Wakade, A.R., 1981. Studies on secretion of catecholamines evoked by acetylcholine or transmural stimulation of the rat adrenal gland. J. Physiol. 313, 463–480.

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.

Wenger, B.W., Bryant, D.L., Boyd, R.T., McKay, D.B., 1997. Evidence for spare nicotinic acetylcholine receptors and a beta 4 subunit in bovine adrenal chromaffin cells: studies using bromoacetylcholine, epibatidine, cytisine and mAb35. J. Pharmacol. Exp. Ther. 281, 905–913.

Xiao, Y., Meyer, E.L., Thompson, J.M., Surin, A., Wroblewski, J., Kellar, K.J., 1998. Rat alpha3/beta4 subtype of neuronal nicotinic acetylcholine receptor stably expressed in a transfected cell line: pharmacology of ligand binding and function. Mol. Pharmacol. 54, 322–333.

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., 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.

Yokotani, K., Okada, S., Murakami, Y., Nakamura, K., 2001. Nicotinic receptors involved in gastric noradrenaline release evoked by electrical stimulation of the splanchnic nerve in rats. Eur. J. Pharmacol. 423, 149–155.