ELSEVIER

European Journal of Pharmacology 319 (1997) 123–130

ejp

Tacrine inhibits nicotinic secretory and current responses in adrenal chromaffin cells

Takeshi Sugawara, Toshio Ohta, Tadashi Asano, Shigeo Ito, Yoshikazu Nakazato *

Laboratory of Pharmacology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

Received 1 October 1996; accepted 18 October 1996

Abstract

Tacrine enhanced acetylcholine-induced catecholamine secretion with a concentration of up to 10 μM, but inhibited it at over 10 μM in perfused adrenal glands. Qualitatively the same result was obtained with physostigmine. Both tacrine and physostigmine only inhibited the secretory responses to carbachol and/or nicotine in perfused glands and dispersed chromaffin cells. Acetylcholinesterase activity of adrenal homogenates was inhibited by tacrine and physostigmine in a concentration-dependent manner. In whole-cell patch-clamp experiments, tacrine and physostigmine caused reversible inhibition of nicotine-evoked inward currents with a dose range similar to that for the inhibitory action on the secretory response. These results suggest that the enhancing effect of tacrine and physostigmine on acetylcholine-induced catecholamine secretion results from the prevention of enzymatic hydrolysis of acetylcholine in adrenal glands and that the inhibitory effect is due to the inhibition of nicotinic receptor-mediated membrane currents in adrenal chromaffin cells.

Keywords: Acetylcholine; Acetylcholinesterase activity; Adrenal homogenate; Nicotine-induced inward current; Physostigmine; Patch clamp

1. Introduction

Pharmacological interest has been focused on 9-amino-1,2,3,4-tetrahydroacridine (THA, tacrine) since tacrine was reported to improve the cognitive function of patients with Alzheimer's disease (Summers et al., 1986; Sahakian et al., 1993). The improvement of Alzheimer's disease symptoms by tacrine has been mainly attributed to its anti-cholinesterase action (Drukarch et al., 1987). On the other hand, tacrine has been demonstrated to inhibit voltage-dependent ionic channels for K^+ and Ca^{2+} in cardiac myocytes (Osterrieder, 1987), for K^+ in hippocampal neurones (Rogawski, 1987; Stevens and Cotman, 1987), for Na^+ and K^+ in frog nerve fibres (Elinder et al., 1989) and for Ca^{2+} in nodose and dorsal root ganglion cells (Kelly et al., 1991). In addition, tacrine and/or physostigmine were shown to inhibit the binding of ligands to muscarinic or nicotinic receptors (Nilsson et al., 1987; Perry et al., 1988; Flynn and Mash, 1989; Nielsen et al., 1989).

In cultured bovine adrenal chromaffin cells, acetylcholine-induced noradrenaline release was increased by physostigmine at concentrations of 10 μM or less and inhibited at higher concentrations in 9–11-day culture, but it was only inhibited dose dependently when acetylcholinesterase activity of the cells was low in earlier culture (Mizobe and Livett, 1982). The stimulatory effect of physostigmine on the response to acetylcholine could be attributable to the anti-cholinesterase action, whereas the inhibitory effects are thought to be due to an interaction with the nicotinic receptor-ionophore complex. Recently, it has been reported that physostigmine inhibits nicotine-induced dopamine release from rat striatal synaptosomes by blocking nicotinic receptors in an insurmountable and pharmacologically selective manner, but tacrine inhibits not only the releasing response to nicotine but also the response to high K^+ (Clarke et al., 1994). Thus, the mechanism underlying the inhibitory action seems to be different between tacrine and physostigmine.

The purpose of the present experiments is to examine the mechanisms of the actions of tacrine on catecholamine secretion and membrane currents evoked by cholinergic agonists compared with those of physostigmine in perfused adrenal glands and dispersed adrenal chromaffin cells of the guinea pig.

* Corresponding author. Tel.: (81-11) 706-5219; Fax: (81-11) 717-7569; e-mail: nakazato@vetmed.hokudai.ac.jp

0014-2999/97/$17.00 Copyright © 1997 Elsevier Science B.V. All rights reserved.
PII S0014-2999(96)00835-7

2. Materials and methods

2.1. Experiments with perfused adrenal glands

Guinea pigs of either sex, weighing 320–400 g, were intraperitoneally anaesthetized with sodium pentobarbitone (40 mg/kg). Both adrenal glands were perfused and isolated following the general procedure reported previously (Ito et al., 1979). The glands were perfused at a flow rate of 0.5–0.6 ml/min by a peristaltic pump at room temperature (approximately 25°C). The adrenal effluent was collected continuously in 5-min aliquots into glass tubes. Experiments were started about 45 min after isolation of adrenal glands. Secretagogues were administered to the glands for 1 min beginning 2 min before the 5-min collection periods because of the 2-min dead time of the arterial cannula. Samples collected were acidified with 8 M perchloric acid to a final concentration of 0.4 M and stored on ice until assayed.

Adrenaline and noradrenaline contained in samples were separated by high-performance liquid chromatography (HPLC, Jasco) and detected by an electrochemical detector (840-EC, Jasco or EC-100, EICOM).

The standard perfusion medium was Locke solution of the following composition (mM): NaCl 154, KCl 5.6, $CaCl_2$ 2.2, Na_2HPO_4-NaH_2PO_4 buffer (pH 7.0) 3 and glucose 10. All solutions were continuously bubbled with pure O_2 throughout the experiments.

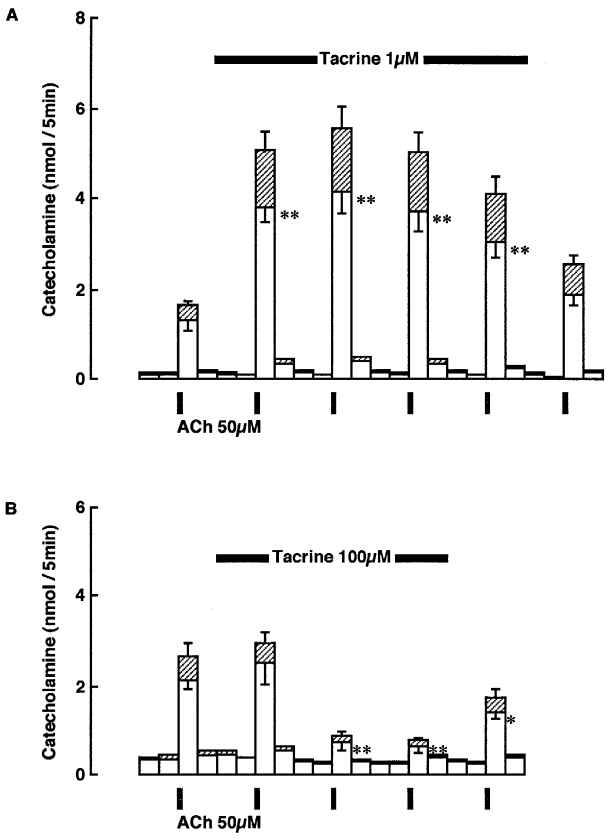

Fig. 1. Effect of tacrine on catecholamine secretion sequentially evoked by acetylcholine in perfused adrenal glands. Acetylcholine (ACh, 50 μM) was applied at times indicated by vertical bars for 1 min at 20-min intervals and tacrine (1 μM in A and 100 μM in B) was infused during the period indicated by horizontal heavy lines above the records. Columns show the mean amounts (and, if vertical lines are present, S.E.M.) of adrenaline (open) and noradrenaline (hatched) released in nmol/5 min obtained from 4 experiments. * $P < 0.05$, ** $P < 0.01$, statistically different from the control value.

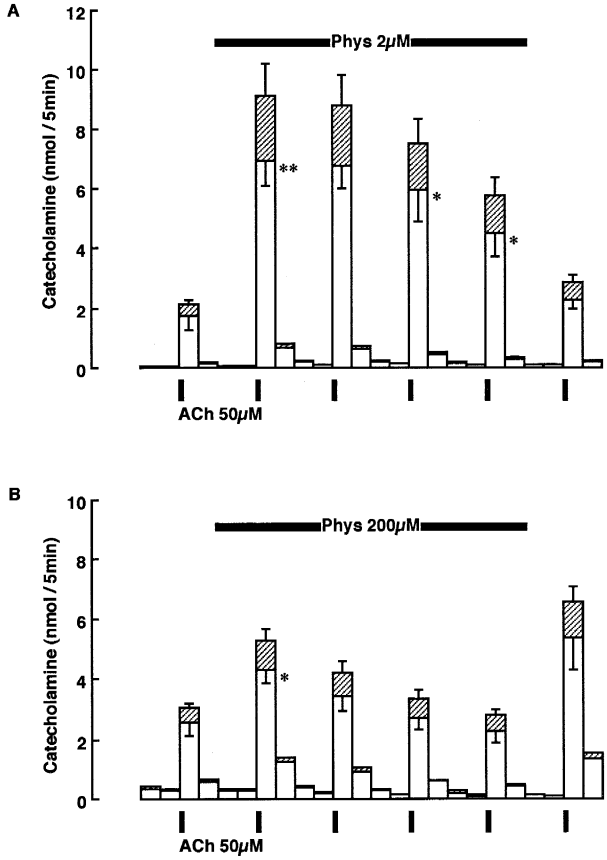

Fig. 2. Effect of physostigmine on catecholamine secretion sequentially evoked by acetylcholine in perfused adrenal glands. Acetylcholine (ACh, 50 μM) was applied at times indicated by vertical bars for 1 min at 20-min intervals and physostigmine (Phys, 2 μM in A and 200 μM in B) was infused during the period indicated by horizontal heavy lines above the records. Columns show the mean amounts (and, if vertical lines are present, S.E.M.) of adrenaline (open) and noradrenaline (hatched) released in nmol/5 min obtained from 4 experiments. * $P < 0.05$, ** $P < 0.01$, statistically different from the control value.

2.2. Measurement of acetylcholinesterase activity of adrenal gland tissues

Guinea pigs of either sex weighing 380–610 g were stunned and bled to death. Adrenal glands were isolated and freed from surrounding tissues in Locke solution containing 1.2 mM Mg^{2+} and then sliced with a razor blade. Sliced adrenal glands were homogenized and the supernatant was stored in ice. The acetylcholinesterase activity of the supernatant was measured following the

method of Ellman et al. (1961) with slight modification in the presence and absence of various concentrations (0.1 nM ~ 1 mM) of tacrine or physostigmine.

2.3. Experiments with dispersed chromaffin cells

Guinea pigs of either sex weighing 250–400 g were stunned and bled to death. Adrenal glands were isolated and freed from surrounding tissues in Ca^{2+}-free physiological salt solution (PSS) and then sliced with a razor blade. Sliced adrenal glands were incubated with Ca^{2+}-free PSS containing collagenase (1 mg/ml) and bovine serum albumin (5 mg/ml) according to the methods of Hochman and Perlman (1976). The isolated chromaffin cells were suspended in PSS (1 ml) containing bovine serum albumin (5 mg/ml) and incubated in a shaking bath (150 oscillations/minute) with drugs at 37°C for 20 min. Cell suspension was then rapidly chilled on ice and centrifuged. The supernatant and pellet were acidified with perchloric acid giving a final concentration of 0.4 M and centrifuged separately. The clear supernatants were used for the assay of catecholamine. Catecholamine release from the isolated cells was expressed as a percentage of catecholamine content in the cells. The PSS contained (mM) NaCl 134, KCl 6, HEPES 10 (pH 7.2, adjusted with 1 M NaOH), $CaCl_2$ 1.7, $MgCl_2$ 1.2 and glucose 10.

2.4. Membrane current recording

Dispersed chromaffin cells were transferred to a small chamber (volume of about 0.4 ml) on the stage of an inverted interference microscope (MD-MSD, Nikon). The cells were superfused with the PSS at a flow rate of 2

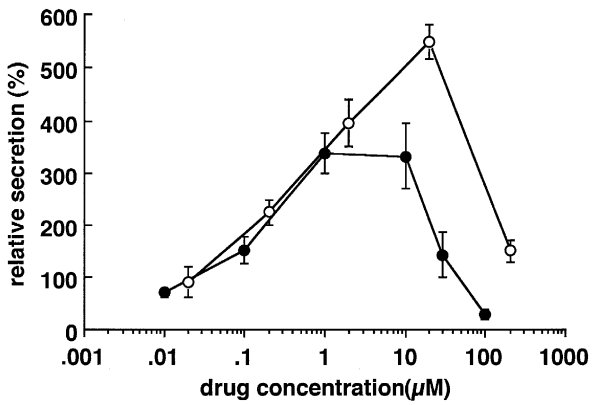

Fig. 3. Concentration-dependent effects of tacrine and physostigmine on catecholamine secretion evoked by acetylcholine in perfused adrenal glands. In experiments using the same protocols as those shown in Figs. 1 and 2, the concentrations of tacrine (●) and physostigmine (○) varied from 0.01 μM to 200 μM. The magnitude of the third secretory response to acetylcholine was expressed as the percentage of that obtained in the absence of either tacrine or physostigmine and was plotted against their concentrations. Symbols indicate the mean of normalized values (and, if vertical lines are present, S.E.M.) of total catecholamine (adrenaline plus noradrenaline) released in nmol/5 min obtained from 4 or 5 experiments.

Table 1
The effect of tacrine and physostigmine on the concentration-dependent increase in catecholamine secretion induced by acetylcholine

ACh (μM)	Control	Tacrine		Physostigmine	
	A + NA	A + NA	Ratios	A + NA	Ratios
1	–	0.39 ± 0.08	–	0.66 ± 0.12	–
10	0.32 ± 0.07	2.80 ± 0.22	8.8	4.49 ± 0.74	14.0
50	1.30 ± 0.10	5.55 ± 0.51	4.3	12.26 ± 2.73	9.4
100	1.80 ± 0.19	7.54 ± 0.74	4.2	14.22 ± 3.71	7.9
500	5.30 ± 0.45	12.03 ± 1.64	2.3	16.83 ± 4.75	3.2

Acetylcholine ranging from 1 to 500 μM was applied for 1 min at 20 min interevals in the absence (Control) and presence of tacrine (1 μM) or physostigmine (2 μM) to single perfused adrenal glands. The values represent catecholamine (adrenaline plus noradrenaline, A + NA) released by acetylcholine (ACh) in nmol/5 min and the ratios of the mean values of the secretory response obtained in the presence of tacrine or physostigmine to those of the respective control. The resting level of catecholamine secretion immediately before the secretory response to acetylcholine was subtracted. The numbers of experiment were 3 for control, 4 for tacrine and 3 for physostigmine.

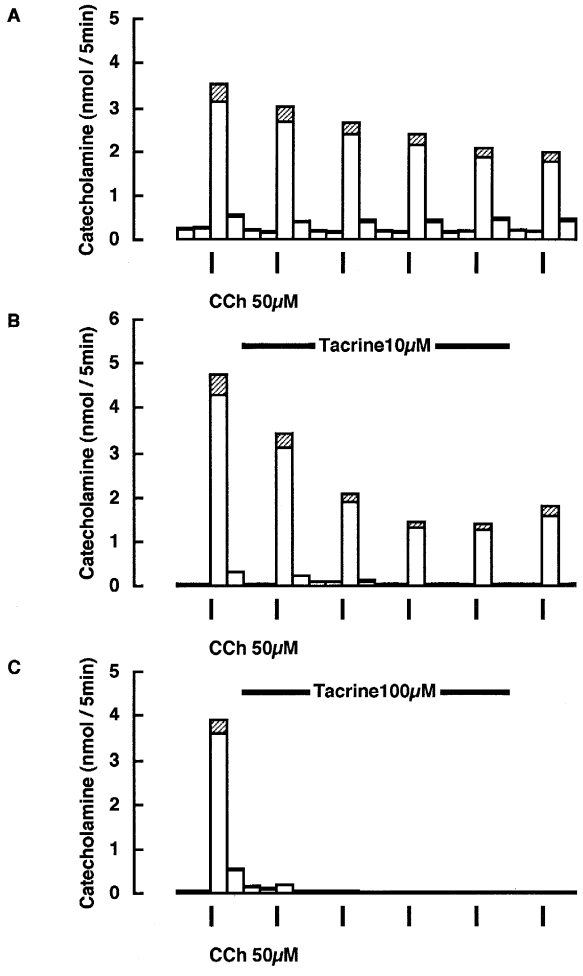

Fig. 4. Effect of tacrine on catecholamine secretion sequentially evoked by carbachol in perfused adrenal glands. Carbachol (CCh, 50 μM) was applied at times indicated by vertical bars for 1 min at 20-min intervals (A), and tacrine (10 μM in B and 100 μM in C) was infused during the period indicated by horizontal heavy lines above the records. Columns show the amounts of adrenaline (open) and noradrenaline (hatched) released in nmol/5 min. Representative experiments are shown.

ml/min and waste fluid was continuously drained. The whole-cell membrane currents were recorded according to conventional patch-clamp techniques (Hamill et al., 1981) at room temperature. The currents were amplified (CEZ-2300, Nihon-Koden) and displayed on an oscilloscope (VC10, Nihon-Koden) and a thermal arraycorder (WR7800, GRAFTEC). Data were stored on a video tape through a PCM data recording system (RF-880, NF Electronic Instruments).

The PSS was also used as the external medium for whole-cell recording. The patch pipette contained (mM): KCl 134, $MgCl_2$ 1.2, glucose 14, HEPES 10, EGTA 10 and ATP 1 (pH 7.2, adjusted with 1 M KOH).

2.5. Drugs and chemicals

Drugs used were: acetylcholine chloride (Ovisort; Daiichi), adenosine-5′-triphosphate disodium salt (ATP, Boehringer Mannheim), *l*-adrenaline bitartrate, *l*-noradrenaline bitartrate, nicotine bi-*l*-(+)-tartrate (Tokyo Kasei), 9-amino-1,2,3,4-tetrahydroacridine (THA, tacrine), bovine serum albumin (Sigma), glycoletherdiamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES) (Dojindo Laboratories) and physostigmine hemisulfate (Eserine, Wako).

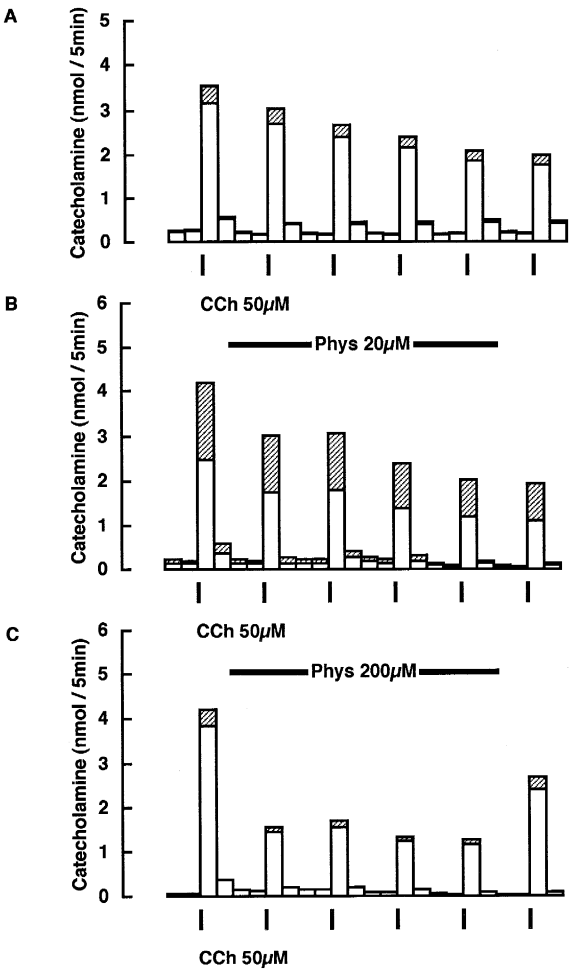

Fig. 5. Effect of physostigmine on catecholamine secretion sequentially evoked by carbachol in perfused adrenal glands. Carbachol (CCh, 50 μM)-induced secretory response in A is the same as that shown in Fig. 4A and physostigmine (Phys, 20 μM in B and 200 μM in C) was infused during the period indicated by horizontal heavy lines above the records. Columns show the amounts of adrenaline (open) and noradrenaline (hatched) released in nmol/5 min. Representative experiments are shown.

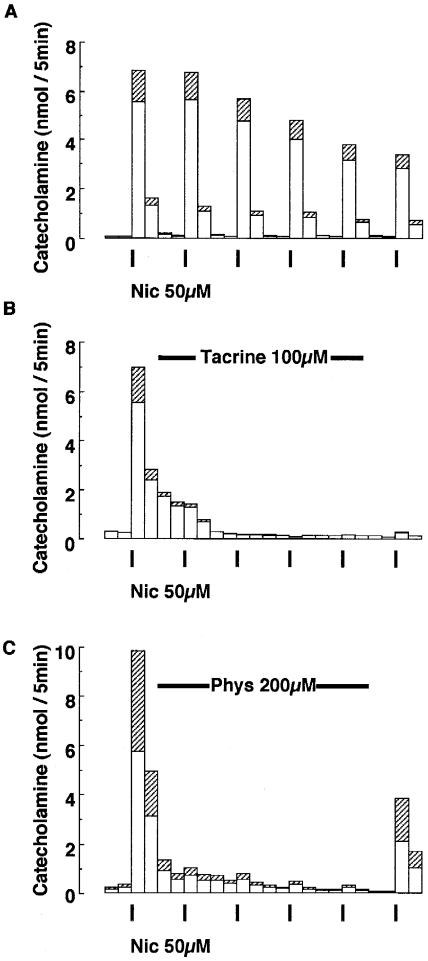

Fig. 6. Effects of tacrine and physostigmine on catecholamine secretion evoked by nicotine in perfused adrenal glands. Nicotine (Nic, 50 μM) was applied at times indicated by vertical bars for 1 min at 20-min intervals (A), and tacrine (100 μM in B) or physostigmine (Phys, 200 μM in C) was infused during the period indicated by horizontal heavy lines above the records. Columns show the amounts of adrenaline (open) and noradrenaline (hatched) released in nmol/5 min. Representative experiments are shown.

2.6. Statistics

The data are presented as arithmetic mean ± S.E.M., and statistical significance was assessed using either Student's *t*-test or the Cochran-Cox test. *P* values of less than 0.05 were considered to be significant.

3. Results

3.1. Effects of tacrine and physostigmine on catecholamine secretion induced by acetylcholine

The effect of tacrine on catecholamine secretion sequentially evoked by acetylcholine (50 μM) was investigated in perfused adrenal glands. Acetylcholine-induced catecholamine secretion was increased to 3–4-fold that of the control at 10 min after the start of application of tacrine (1 μM) and gradually declined thereafter, though the magnitude of the response was still about 3-fold that of the control at 70 min after the start of infusion of tacrine (Fig. 1A). There was no appreciable difference in the effects on adrenaline and noradrenaline secretions and, therefore, we expressed them only as catecholamine unless otherwise noted below.

When a high concentration of tacrine such as 100 μM was applied, the secretory response to acetylcholine was about the same size as that of the control at 10 min, and then it decreased to one-third of the control level at 30 min after the start of application (Fig. 1B). The inhibitory effect was restored after withdrawal of tacrine. As tacrine has been reported to possess potent anti-cholinesterase activity (Heilbronn, 1961), the effect of physostigmine on acetylcholine-induced responses was observed and compared with that of tacrine.

Similar to tacrine, physostigmine (2 μM) also enhanced both adrenaline and noradrenaline secretions induced by acetylcholine (Fig. 2A). The magnitude of the enhancement was 4–5.5-fold that of the control at 10 min and gradually declined thereafter. When the concentration of physostigmine was increased to 200 μM, the enhancing effect was greatly decreased so that the maximal response was only about 150% of the control level at 10 min after the start of application of physostigmine (Fig. 2B). Then the acetylcholine-induced secretory response gradually declined until it attained about the same size as the control at 70 min. After removal of physostigmine, however, the response to acetylcholine was enhanced to more than 2-fold that of the control.

To examine the concentration-effect relationships for tacrine and physostigmine, we varied the concentrations of both drugs in the experiment using the same protocols as those shown in Figs. 1 and 2, and plotted the magnitude of the third secretory response to acetylcholine in Fig. 3. The enhancing effects started to appear at 0.1–0.2 μM, and attained maxima of $336.5 \pm 32.8\%$ ($n = 4$) at 1 μM tacrine and $549.9 \pm 32.4\%$ ($n = 4$) at 20 μM physostigmine. A similar magnitude of the enhancing effect was still observed at 10 μM tacrine, but it disappeared or turned to inhibition over this concentration. The enhancing effect of physostigmine was also depressed over 20 μM.

Next, the enhancing effect of tacrine or physostigmine on the secretory responses to various concentrations of acetylcholine was studied. Acetylcholine from 1 to 500 μM was sequentially applied for 1 min at 20 min intervals to single perfused adrenal glands. The secretory response to acetylcholine started to occur at 10 μM and increased in magnitude by increasing its concentration to 500 μM. In the glands treated with either tacrine or physostigmine, the secretory response to acetylcholine started to appear at 1 μM and the magnitude of the responses was significantly enhanced as compared with that obtained in untreated glands (Table 1). The ratios of the enhanced response in the presence of either tacrine or physostigmine to the respective control were inversely proportional to the concentration of acetylcholine (Table 1).

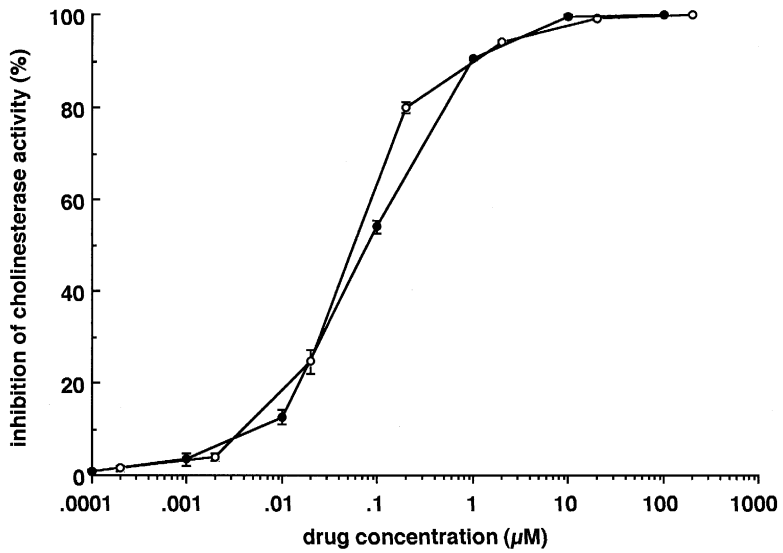

Fig. 7. Concentration-dependent inhibition of acetylcholinesterase activity of adrenal homogenates by tacrine and physostigmine. Symbols indicate the mean percent inhibition (and, if vertical lines are present, S.E.M.) of acetylcholinesterase activity by various concentrations of tacrine (●) and physostigmine (○) from 0.1 nM to 200 μM. The number of experiments was 4.

There was no detectable effect of either drug on the basal catecholamine secretion.

3.2. Effects of tacrine and physostigmine on catecholamine secretion evoked by carbachol and nicotine

If the enhancing effects of tacrine and physostigmine on acetylcholine-induced secretory response resulted from the inhibition of acetylcholinesterase activity, neither drugs should be effective in enhancing the response to carbachol or nicotine, which are not substrates for acetylcholinesterase. Fig. 4 shows that tacrine slightly inhibited carbachol-induced responses at 10 μM and abolished them at 100 μM. Similar results were obtained with physostigmine (Fig. 5). Nicotine-induced catecholamine secretion was also greatly inhibited by tacrine (100 μM) and physostigmine (200 μM) (Fig. 6), but was not affected by the low concentration of the drugs. The recovery from the inhibitory effect on the response to nicotine was much greater in the glands treated with physostigmine than in those treated with tacrine.

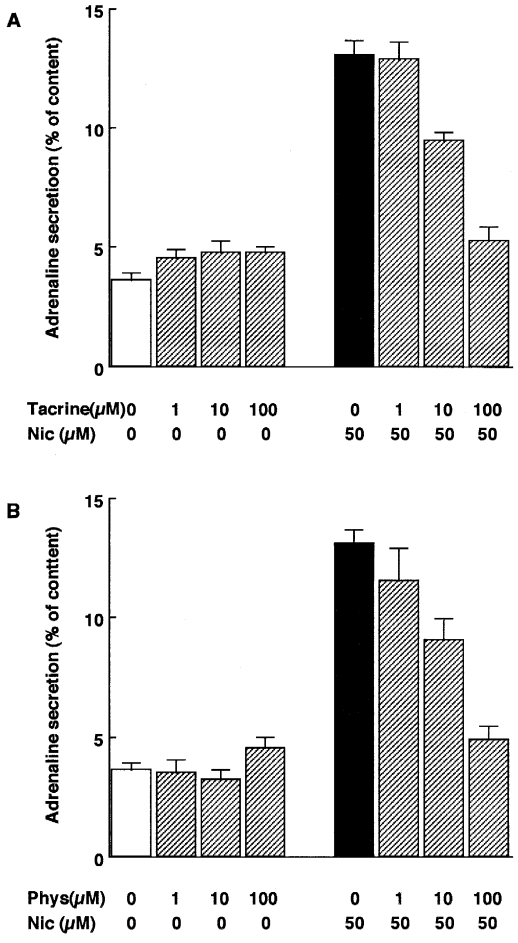

Fig. 8. Concentration-dependent inhibition of nicotine-induced adrenaline secretion in dispersed chromaffin cells. Cells were incubated in the presence and absence of tacrine (A) and physostigmine (Phys) (B) with or without nicotine (Nic) as indicated at the bottom of the records. Columns indicate the mean, with S.E.M. shown by vertical lines, of adrenaline release expressed as the percentage of total cell contents in 10 (open and closed which are the same as in A and B) and 5 (hatched) experiments.

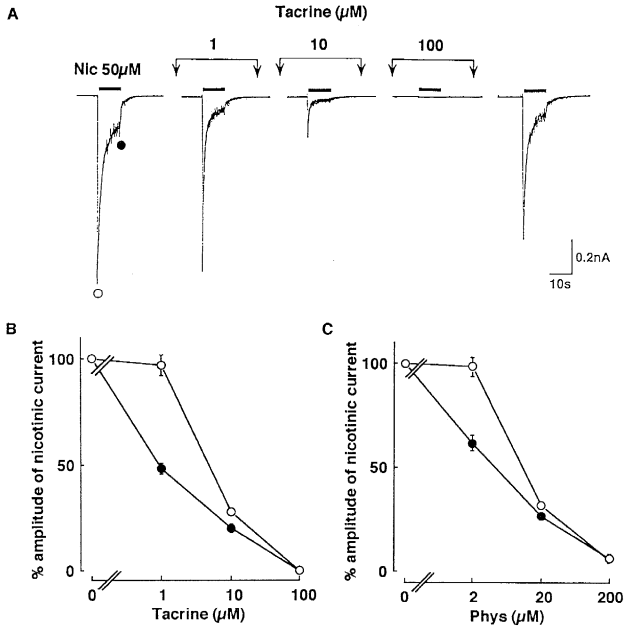

Fig. 9. Inhibitory effect of tacrine and physostigmine on inward currents evoked by nicotine in whole-cell patch-clamped cells. Nicotine (Nic, 50 μM) was sequentially applied for 10 s before and during exposure to tacrine of which the concentration was progressively increased as indicated above the records, and after its washout (A). Concentration-inhibition curves for tacrine (B) and physostigmine (C) are shown. Symbols indicate the mean percent inhibition (and, if vertical lines are present, S.E.M.) of the nicotine-induced initial peak (○) and sustained (●) currents by tacrine and physostigmine from 1 to 200 μM. The number of experiments was 5.

3.3. Inhibitory effects of tacrine and physostigmine on acetylcholinesterase activity of adrenal gland tissues

The inhibition of acetylcholinesterase activity of adrenal gland tissues started to appear at concentrations of 10 ~ 20 nM of either tacrine or physostigmine, increased with increasing concentrations and attained a maximum at 10 ~ 20 μM (Fig. 7). The IC_{50} values were computed to be 89.1 ± 4.8 nM ($n = 4$) for tacrine and 55.7 ± 3.7 nM ($n = 4$) for physostigmine.

3.4. Inhibitory effects of tacrine and physostigmine on nicotine-induced secretory and current responses in dispersed cells

To examine the mechanism of the inhibitory effects of tacrine and physostigmine on the secretory response, we investigated the effects of these drugs on the secretion and membrane current evoked by nicotine in dispersed chromaffin cells.

Cells were incubated for 25 min at 37°C in the presence and absence of tacrine or physostigmine in concentrations ranging from 1 to 100 μM. At 5 min after the start of this

incubation, nicotine (50 μM) was added to the incubation medium, except for the control. After incubation for 20 min, adrenaline released to the incubation medium was measured and expressed as a percentage of total adrenaline content. We did not measure noradrenaline, because of its much lesser content compared to adrenaline in the dispersed cells. As shown in Fig. 8, both tacrine and physostigmine had no detectable effect on the resting adrenaline secretion, but caused concentration-dependent inhibition of nicotine-induced adrenaline secretion. The IC_{50} values for both drugs were almost the same, 13.9 ± 2.7 μM ($n = 4$) for tacrine and 13.3 ± 2.2 μM ($n = 5$) for physostigmine.

Nicotine-induced membrane current was studied using the whole-cell patch-clamp technique. At a holding potential of −60 mV, nicotine (50 μM) evoked an inward current consisting of an initial transient peak (844.8 ± 113.5 pA, $n = 10$) and a later sustained phase (297.6 ± 41.1 pA, $n = 10$) in all cells tested. In a representative result shown in Fig. 9A, tacrine caused a concentration-dependent inhibition of the inward current evoked by nicotine and the inhibition was easily restored after withdrawal of tacrine. The sustained current was slightly more sensitive to tacrine than the initial peak current (Fig. 9B). Similarly, physostigmine reversibly inhibited nicotinic current in a concentration-dependent manner (Fig. 9C). The IC_{50} values for tacrine were 4.9 ± 0.7 μM and 3.9 ± 1.8 μM ($n = 5$), and those for physostigmine 10.8 ± 1.8 μM and 5.5 ± 0.8 μM ($n = 5$), in inhibiting the peak and sustained currents, respectively.

4. Discussion

Various mechanisms of action of tacrine on transmitter release have been reported. For example, tacrine augments neuromuscular transmission resulting from the inhibition of acetylcholinesterase (Braga et al., 1991), stimulates the spontaneous secretion of large quanta of acetylcholine at motor nerve terminals (Thesleff et al., 1990) and displaces noradrenaline from intraneuronal transmitter stores of sympathetically innervated tissues (Fabiani et al., 1992). The present experiments showed that tacrine caused enhancement of acetylcholine-induced catecholamine secretion with lower concentrations and inhibition with higher concentrations in perfused adrenal glands of the guinea pig. The enhancing effect of tacrine was considered to be attributed to its anti-acetylcholinesterase action as reported by Heilbronn (1961) because of the following observations. First, tacrine and physostigmine showed a similar concentration-dependent increase in the acetylcholine-induced secretory response. Both tacrine and physostigmine enhanced to a much larger extent the secretory responses to the lower concentration of acetylcholine than those to its higher concentration. Second, the secretory responses to carbachol and nicotine, which are not substrates for cholinesterase, were not enhanced. Third, tacrine caused the same concentration-dependent inhibition of acetylcholinesterase activity of adrenal tissue homogenates as physostigmine.

In the present experiments, both tacrine and physostigmine also caused inhibition of catecholamine secretion induced by cholinergic agonists. According to Clarke et al. (1994), physostigmine inhibits nicotine-induced dopamine release from rat striatal synaptosomes by blocking nicotinic receptors in an insurmountable and pharmacologically selective manner, but tacrine inhibits not only nicotine-induced dopamine release, but also that induced by high K^+. On the other hand, tacrine and/or physostigmine have been reported to inhibit voltage-dependent Na^+ channels in giant axons (Schauf and Sattin, 1987) and at neuromuscular junction (Elinder et al., 1989), K^+ channels in hippocampal neurones (Rogawski, 1987), snail neurones (Drukarch et al., 1987), atrial muscle (Freeman et al., 1988) and cardiac myocytes (Osterrieder, 1987), and Ca^{2+} channels in cardiac myocytes (Osterrieder, 1987) and nodose and dorsal root ganglion cells (Kelly et al., 1991). In dispersed chromaffin cells, we found that both catecholamine secretion and inward current evoked by nicotine were inhibited by either tacrine or physostigmine with similar IC_{50} values. It seems, therefore, likely that the inhibition of the inward current is the primary cause of the inhibitory actions of tacrine and physostigmine on the secretory response. This inhibitory effect must be independent of the ability to inhibit acetylcholinesterase (Clarke et al., 1994), because the IC_{50} values for both drugs in inhibiting the nicotine-induced secretory response and acetylcholinesterase activity are considerably different.

Tacrine and/or physostigmine have also been demonstrated to weakly inhibit the binding of ligands to the nicotinic or muscarinic receptor in brain tissues (Nilsson et al., 1987; Perry et al., 1988; Nielsen et al., 1989; Flynn and Mash, 1989). The difference in the recovery rate between tacrine- and physostigmine-mediated inhibition of the secretory response to nicotine could be due to the difference in their binding affinity to nicotinic receptor. However, this is unlikely in the present experiment, because the inhibitory effect on nicotine-induced inward current was easily restored after withdrawal of either tacrine or physostigmine. Furthermore, we do not know whether the inhibition of inward current results from the inhibition of the binding of nicotine to the receptor or a direct effect on nicotinic channel itself at present. Therefore, the mechanism of the inhibitory action of tacrine and physostigmine has to be studied further in detail in future.

Acknowledgements

This work was partly supported by grants from the Japanese Ministry of Education, Science and Culture and the Akiyama Foundation.

References

Braga, M.F.M., A.L. Harvey and E.G. Rowan, 1991, Effects of tacrine, velnacrine (HP029), suronacrine (HP128) and 3,4-diaminopyridine on skeletal neuromuscular transmission in vitro, Br. J. Pharmacol. 102, 909.

Clarke, P.B.S., M. Reuben and H. El-Bizri, 1994, Blockade of nicotinic responses by physostigmine, tacrine and other cholinesterase inhibitors in rat striatum, Br. J. Pharmacol. 111, 695.

Drukarch, B., K.S. Kits, E.G. Van der Meer, J.C. Lodder and J.C. Stoof, 1987, 9-Amino-1,2,3,4-tetrahydroacridine (THA), an alleged drug for the treatment of Alzheimer's disease, inhibits acetylcholinesterase activity and slow outward K^+ current, Eur. J. Pharmacol. 141, 153.

Elinder, F., A.K. Mohammed, B. Winblad and P. Arhem, 1989, Effects of THA on ionic currents in myelinated axons of *Xenopus laevis*, Eur. J. Pharmacol. 164, 599.

Ellman, G.L., K.D. Courtney, V. Andres Jr. and R.M. Featherstone, 1961, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7, 88.

Fabiani, M.E., P. Kabo and O.F. Story, 1992, Prejunctional actions of tacrine on autonomic neuroeffector transmission in rabbit isolated pulmonary artery and rat isolated atria, Clin. Exp. Pharmacol. Physiol. 19, 631.

Flynn, D.D. and D.C. Mash, 1989, Multiple in vitro interactions with and differential in vivo regulation of muscarinic receptor subtypes by tetrahydroaminoacridine, J. Pharmacol. Exp. Ther. 250, 573.

Freeman, S.E., W.-M. Lau and M. Szilagyi, 1988, Blockade of a cardiac K^+ channel by tacrine: interactions with muscarinic and adenosine receptors, Eur. J. Pharmacol. 154, 59.

Hamill, O.P., A. Marty, E. Neher, B. Sakmann and F.J. Sigworth, 1981, Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches, Pflüg. Arch. 391, 85.

Heilbronn, E., 1961, Inhibition of cholinesterases by tetrahydroaminoacridine, Acta Chem. Scand. 15, 1386.

Hochman, J. and R.L. Perlman, 1976, Catecholamine secretion by isolated adrenal cells, Biochim. Biophys. Acta 421, 168.

Ito, S., Y. Nakazato and A. Ohga, 1979, The effect of veratridine on the release of catecholamines from the perfused adrenal gland, Br. J. Pharmacol. 65, 319.

Kelly, K.M., R.A. Gross and R.L. Macdonald, 1991, Tetrahydroaminoacridine (THA) reduces voltage-dependent calcium currents in rat sensory neurons, Neurosci. Lett. 132, 247.

Mizobe, F. and B.G. Livett, 1982, Biphasic effect of eserine and other acetylcholinesterase inhibitors on the nicotinic response to acetylcholine in cultured adrenal chromaffin cells, J. Neurochem. 39, 379.

Nielsen, J.A., E.E. Mena, I.H. Williams, M.R. Nocerini and D. Liston, 1989, Correlation of brain levels of 9-amino-1,2,3,4-tetrahydroacridine (THA) with neurochemical and behavioral changes, Eur. J. Pharmacol. 173, 53.

Nilsson, L., A. Adem, J. Hardy, B. Winblad and A. Nordberg, 1987, Do tetrahydroaminoacridine (THA) and physostigmine restore acetylcholine release in Alzheimer brains via nicotinic receptors? J. Neural Transm. 70, 357.

Osterrieder, W., 1987, 9-Amino-1,2,3,4-tetrahydroacridine (THA) is a potent blocker of cardiac potassium channels, Br. J. Pharmacol. 92, 521.

Perry, E.K., C.J. Smith, J.A. Court, J.R. Bonham, B.M. Rodway and J.R. Atack, 1988, Interaction of 9-amino-1,2,3,4-tetrahydroamino-acridine (THA) with human cortical nicotinic and muscarinic receptor binding in vitro, Neurosci. Lett. 91, 211.

Rogawski, M.A., 1987, Tetrahydroaminoacridine blocks voltage-dependent ion channels in hippocampal neurons, Eur. J. Pharmacol. 142, 169.

Sahakian, B.J., A.M. Owen, N.J. Morant, S.A. Eagger, S. Boddington, L. Crayton, H.A. Crockford, M. Crooks, K. Hill and R. Levy, 1993, Further analysis of the cognitive effects of tetrahydroamino-acridine (THA) in Alzheimer's disease: assessment of attentional and mnemonic function using CANTAB, Psychopharmacology 110, 395.

Schauf, C.L. and A. Sattin, 1987, Tetrahydroaminoacridine blocks potassium channels and inhibits sodium inactivation in *Myxicola*, J. Pharmacol. Exp. Ther. 243, 609.

Stevens, D.R. and C.W. Cotman, 1987, Excitatory actions of tetrahydro-9-aminoacridine (THA) on hippocampal pyramidal neurons, Neurosci. Lett. 79, 301.

Summers, W.K., L.V. Majovski, G.M. Marsh, K. Tachiki and A. Kling, 1986, Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type, New Engl. J. Med. 315, 1241.

Thesleff, S., L.C. Sellin and S. Taggerud, 1990, Tetrahydroamino-acridine (tacrine) stimulates neurosecretion at mammalian motor endplates, Br. J. Pharmacol. 100, 487.