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Influence of Tacrine on Catecholamine Secretion in the Perfused Rat Adrenal Gland

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The present study was designed to clarify whether tacrine affects the release of catecholamines (CA) from the isolated perfused model of rat adrenal gland or not and to elucidate the mechanism of its action. Tacrine ($3\times10^{-5}\sim3\times10^{-4}$ M) perfused into an adrenal vein for 60 min inhibited CA secretory responses evoked by ACh (5.32×10^{-3} M), DMPP (a selective neuronal nicotinic agonist, 10^{-4} M for 2 min) and McN-A-343 (a selective muscarinic M_1-agonist, 10^{-4} M for 2 min) in relatively dose- and time-dependent manners. However, tacrine failed to affect CA secretion by high K^+ (5.6×10^{-2} M). Tacrine itself at concentrations used in the present experiments did not also affect spontaneous CA output. Furthermore, in the presence of tacrine (10^{-4} M), CA secretory responses evoked by Bay-K-8644 (an activator of L-type Ca^{2+} channels, 10^{-4} M), but not by cyclopiazonic acid (an inhibitor of cytoplasmic Ca^{2+}-ATPase, 10^{-4} M), was relatively time-dependently attenuated. Also, physostigmine (10^{-4} M), given into the adrenal gland for 60 min, depressed CA secretory responses evoked by ACh, McN-A-343 and DMPP while did not affect that evoked by high K^+. Collectively, these results obtained from the present study demonstrate that tacrine greatly inhibits CA secretion from the perfused rat adrenal gland evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors, but does fail to affect that by direct membrane-depolarization. It is suggested that this inhibitory effect of tacrine may be exerted by blocking both the calcium influx into the rat adrenal medullary chromaffin cells without Ca^{2+} release from the cytoplasmic calcium store, that is relevant to the cholinergic blockade. Also, the mode of action between tacrine and physostigmine in rat adrenomedullary CA secretion seems to be similar.

Key Words: Tacrine, Physostigmine, Perfused adrenal gland, Catecholamine secretion

INTRODUCTION

Tacrine (9-amino-1,2,3,4-tetrahydroacridine, THA) is a long-lasting, potent, centrally active, reversible inhibitor of acetylcholinesterase and also has some central cholinomimetic properties (Sunaga et al, 1993; Szilagyi & Lau, 1993; Xiao et al, 1993). Tacrine has been reported to improve cognitive and memory functions in patients with Alzheimer's disease (Summers et al, 1986; Eagger et al, 1991; Davis et al, 1992; Farlow et al, 1992; Sahakian & Coull, 1993; Sahakian et al, 1993; Knapp et al, 1994;), and to decrease the veratridine-induced secretion of catecholamines (CA) primarily by inhibiting the voltage-dependent Na^+ channels rather than the Ca^{2+} channels in guinea-pig adrenal chromaffin cells (Sugawara et al, 1998). Furthermore, it inhibits voltage-dependent ionic channels for K^+ and Ca^{2+} in cardiac myocytes (Osterrieder, 1987), for K^+ in hippocampal neurones (Rogawski, 1987; Stevens & 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).

High concentrations of tacrine have been reported to inhibit the binding of radio-labelled ligands to muscarinic and/or nicotinic receptors (Nilsson et al, 1987; Flynn & Mash, 1989; Kiefer-Day et al, 1991), and to block the inhibition of cyclic AMP formation and the PI-hydrolysis evoked by muscarinic stimulation (Kiefer-Day et al, 1993). Higher concentrations of tacrine and physostigmine inhibit the ACh-induced CA secretion by blockade of nicotinic receptors, whereas lower concentrations enhance such secretions through their anticholinesterase actions (Sugawara et al, 1997). In rat striatal synaptosomes, tacrine inhibits high K^+-evoked dopamine release, but physostigmine does not (Clarke et al, 1994). However, Sugawara and his co-workers (1998) found that the high K^+ (46.2 mM)-evoked CA secreton in guinea-pig adrenal chromaffin cells was not affected by tacrine ($1\sim100\,\mu$M) or physostigmine ($1\,\mu$M~1 mM).

On the other hand, tacrine with a concentration of up to $10\,\mu$M was found to enhance ACh-induced CA secretion in perfused guinea-pig adrenal glands (Sugawara et al,

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ABBREVIATIONS: CA, catecholamines; ACh, acetylcholine; DMPP, 1.1-dimethyl-4-phenyl piperazinium iodide; Bay-K-8644; methyl-1, 4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridin e-5-carboxylate; McN-A-343, (3-(m-chloro-phenyl-carbamoyl-oxy)-2-butynyl trimethyl ammonium chloride.

1997). Furthermore, it has been shown that tacrine enhances monoamine neurotransmission in the rat striatum, probably via an interaction with both muscarinic and nicotinic heteroreceptors (Warpman et al, 1996). More recently, it has been also shown that both intravenouse and intracerebroventricular tacrine stimulates central muscarinic cholinoceptors to increase blood pressure in rats (Savci et al, 1998) and dogs (Allal et al, 1998). Increases in plasma CA and vasopressin are involved in this pressor response. There appears to be a controversy in tacrine-induced pharmacological effects, especially CA secretion-related effects. Therefore, the aim of the present study was to investigate the effect of tacrine on CA releasing responses evoked by stimulation of cholinergic receptors and direct membrane-depolarization in the isolated perfused model of the rat adrenal gland.

METHODS

Experimental procedure

Sprague-Dawley male rats, weighing 180 to 250 grams, were anesthetized with thiopental sodium (40 mg/kg) intraperitoneally. The adrenal gland was isolated by the methods described previously (Wakade, 1981). The abdomen was opened by a midline incision, and the left adrenal gland and surrounding area were exposed by placing three hook retractors. The stomach, intestine and portion of the liver were not removed, but pushed over to the right side and covered by saline-soaked gauge pads. Urine in bladder was removed in order to obtain enough working space for tying blood vessels and cannulations. A cannula, used for perfusion of the adrenal gland, was inserted into the distal end of the renal vein after all branches of adrenal vein (if any), vena cava and aorta were ligated. Heparin (400 IU/ml) was injected into vena cava to prevent blood coagulation before ligating vessels and cannulations. A small slit was made into the adrenal cortex just opposite entrance of adrenal vein. Perfusion of the gland was started after confirming no leakage, and the perfusion fluid escaped only from the slit made in adrenal cortex. Then the adrenal gland, along with ligated blood vessels and the cannula, was carefully removed from the animal and placed on a platform of a leucite chamber. The chamber was continuously circulated with water heated at $37\pm1^{\circ}C$ (Fig. 1).

Perfusion of adrenal gland

The adrenal glands were perfused by means of a peristaltic pump (WIZ Co.) at a rate of 0.3 ml/min. The perfusion was carried out with Krebs-bicarbonate solution of following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.18; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 11.7. The solution was constantly bubbled with 95% O_2+5% CO_2 and the final pH of the solution was maintained at 7.4~7.5. The solution contained disodium EDTA (10 μg/ml) and ascorbic acid (100 μg/ml) to prevent oxidation of CAs.

Drug administration

The perfusions of DMPP (100 μM) and McN-A-343 (100 μM) for 2 minutes, and Bay-K-8644 (10 μM) and cyclopiazonic acid (10 μM) for 4minutes were made into perfusion stream, respectively. A single injection of ACh (5.32 mM) or KCl (56 mM) in a volume of 0.05 ml was made into perfusion stream via a three-way stopcock.

In the preliminary experiments, it was found that, upon administration of the above drugs, secretory responses to ACh, KCl, McN-A-343, Bay-K-8644 and cyclopiazonic acid returned to pre-injection level in about 4 min, but the responses to DMPP in 8 min.

Collection of perfusate

As a rule, prior to stimulation with various secretagogues, perfusate was collected for 4 min to determine the spontaneous secretion of CA (background sample). Immediately after the collection of the background sample, collection of the perfusates was continued in another tube as soon as the perfusion medium containing the stimulatory agent reached the adrenal gland. Stimulated sample's was collected for 4 to 8 min. The amounts in the background sample were subtracted from that secreted from those of stimulated sample to obtain the net secretion of CA, which are shown in all of the following figures.

To study the effects of tacrine on the spontaneous and evoked secretions, the adrenal gland was perfused with Krebs solution containing tacrine for 60 min immediately after the perfusate was collected for a certain period (background sample). Then, the medium was changed to the one containing the stimulating agent, and the perfusates were collected for the same period as that for the background sample. Generally, the perfusates were collected in chilled tubes.

Measurement of catecholamines

CA content of perfusate was measured directly by the method of Anton & Sayre (1962) using fluorospectrophotometer (Kontron Co. Italy) without the intermediate purification on alumina, because of the reasons described earlier (Wakade, 1981).

A 0.2 ml volume of the perfusate was used for the reaction. The CA content in the perfusate of stimulated glands by secretogagues used in the present work was high enough to obtain readings several folds greater than the reading of control samples (unstimulated). The sample blanks were also lowest for perfusates of stimulated and non-stimulated samples. The content of CA in the perfusate was expressed in terms of norepinephrine (base) equivalents.

Statistical analysis

The statistical significance between groups was determined by utilizing the Student's t-test. A P-value of less than 0.05 was considered to represent statistically significant changes, unless specifically noted in the text. Values given in the text refer to means and the standard errors of the mean (S.E.M.). The statistical analysis of the experimental results was made by computer program described by Tallarida & Murray (1987).

Drugs and their sources

Tacrine hydrochloride, acetylcholine chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), norepinephrine bitartrate, nicotine tartrate, methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (BAY-K-8644), physostigmine sulfate were pur-

chased from Sigma Chemical Co., U.S.A. Cyclopiazonic acid and (3-(m-chloro-phenyl-carbamoyl-oxy)-2-butynyl trimethyl ammonium chloride [McN-A-343] were purchased from RBI Co., U.S.A. Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required, except Bay-K-8644 which was dissolved in 99.5% ethanol and diluted appropriately (final concentration of alcohol was less than 0.1%). Concentrations of all drugs used are expressed in molar concentration.

RESULTS

Effect of tacrine on CA secretion from the perfused rat adrenal glands evoked by ACh, high K^+, DMPP and McN-A-343

Recently, it has been found that tacrine decreases the veratridine-induced secretion of catecholamines (CA) primarily by inhibiting the voltage-dependent Na^+ channels rather than the Ca^{2+} channels in guinea-pig adrenal chromaffin cells (Sugawara et al, 1998). Therefore, it was decided first to examine the effects of tacrine on cholinergic receptor stimulation- as well as membrane depolarization-mediated CA secretion from perfused rat adrenal glands. Secretagagues were given at 15 or 20 min-intervals, and tacrine was present for 60 min including stimulation with each secretagogue. In the present study, it was found that tacrine (30~300 μM) itself did not produce any effect on basal CA output (data not shown). Basal CA release from the isolated perfused rat adrenal glands amounted to 22±3 ng/2 min (n=8) after the initial perfusion with oxygenated Krebs-bicarbonate solution for 1 hr.

When ACh (5.32×10^{-3} M) in a volume of 0.05 ml was injected into the perfusion stream, the amounts of CA secreted was 339±34 ng for 4 min. As shown in Fig. 1. However, the pretreatment with tacrine in the range of 3×10^{-5}~3×10^{-4} M for 60 min concentration- and time-dependently inhibited ACh-stimulated CA secretion from adrenal glands by 78~14% of the control (100%), respectively. Also, a depolarizing agent such as KCl strongly stimulated CA secretion (175±12 ng for 0~4 min). However, excess K^+ (5.6×10^{-2} M)-stimulated CA secretion in the presence of tacrine was not affected as compared with its corresponding control secretion (100%) (Fig. 2). When perfused through the rat adrenal gland, DMPP (10^{-4} M for 1 min), which is a selective nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase of CA secretion (478±63 ng for 0~8 min). However, as shown in Fig. 3, DMPP-stimulated CA secretion after pretreatment with tacrine was greatly reduced time-dependently to 81~18% of the corresponding control (100%). McN-A-343 (10^{-4} M), which is a selective muscarinic M_1-agonist (Hammer & Giachetti, 1982), perfused into an adrenal gland for 4 min caused an increased CA secretion (92±12 ng for 0~4 min). However, McN-A-343-stimulated CA secretion in the presence of tacrine was markedly depressed as compared to the corresponding control secretion (Fig. 4).

Effect of tacrine on CA secretion from the perfused rat adrenal glands evoked by Bay-K-8644 and cyclopiazonic acid

Bay-K-8644 is a calcium channel activator which causes positive inotropy and vasoconstriction in isolated tissues

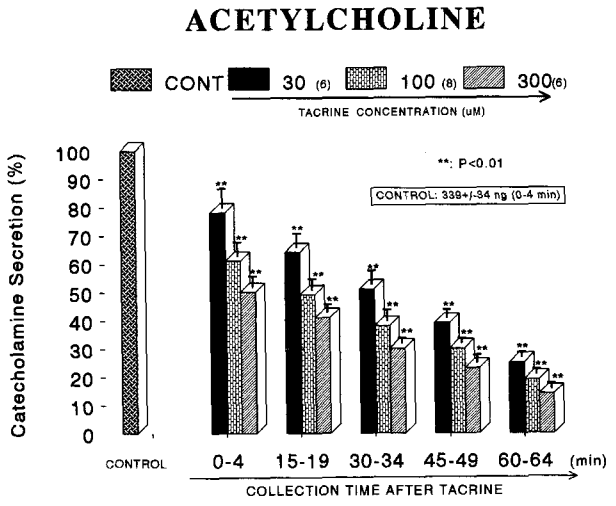

Fig. 1. Dose-dependent effect of tacrine on secretory responses of catecholamines (CA) evoked by acetylcholine (ACh) from the isolated perfused rat adrenal glands. CA secretion by a single injection of ACh (5.32×10^{-3} M) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with 30, 100, 300 M of tacrine for 60 min as indicated by an arrow mark. Numbers in the parenthesis indicate number of rat adrenal glands. Vertical bars on the columns represent the standard error of the mean (S.E.M.). Ordinate: the amounts of CA secreted from the adrenal gland (% of control). Abscissa: collection time of perfusate (min). Statistical difference was obtained by comparing the corresponding control (CONT) with each concentration-pretreated group of tacrine. ACh-induced perfusate was collected for 4 minutes.

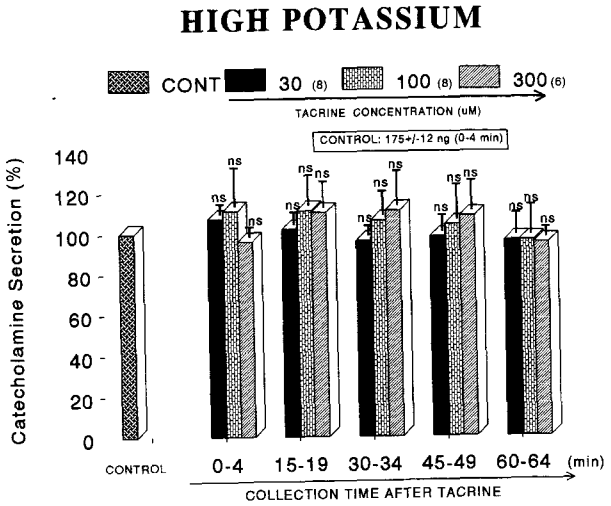

Fig. 2. Dose-dependent effect of tacrine on secretory responses of catecholamines (CA) evoked by high K^+ from the isolated perfused rat adrenal glands. High K^+ (56 mM) in a volume of 0.05 ml was injected at 15min intervals after preloading with 30, 100, 300 μM of tacrine for 60 min. Statistical difference was obtained by comparing the corresponding control with each concentration-pretreated group of tacrine. K^+-induced perfusate was collected for 4 minutes. Other legends are the same as in Fig. 1. ns: Statistically insignificant.

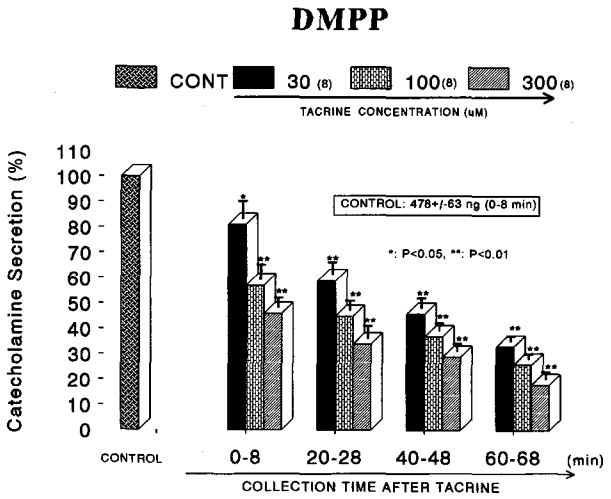

Fig. 3. Dose-dependent effect of tacrine on secretory responses of catecholamines (CA) evoked by DMPP from the isolated perfused rat adrenal glands. DPPP (10^{-4} M) was infused for 2 min at 20 min intervals after preloading with 30, 100, 300 μM of tacrine for 60 min. Statistical difference was obtained by comparing the corresponding control (CONT) with each concentration-pretreated group of tacrine. DMPP-induced perfusate was collected for 8 minutes. Other legends are the same as in Fig. 1.

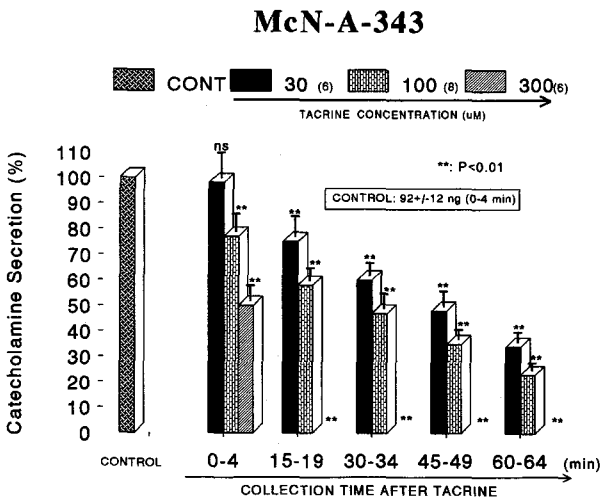

Fig. 4. Dose-dependent effect of tacrine on secretory responses of catecholamines (CA) evoked by McN-A-343 from the isolated perfused rat adrenal glands. McN-A-343 (10^{-4} M) was infused for 4 min at 15 min intervals after preloading with 30, 100, 300 μM of tacrine for 60 min. Statistical difference was obtained by comparing the corresponding control with each concentration-pretreated group of tacrine. McN-A-343-induced perfusate was collected for 4 minutes. Other legends are the same as in Fig. 1. ns: Statistically insignificant.

and intact animals (Schramm et al, 1982; Wada et al, 1985) and enhances basal Ca^{2+} uptake (Garcia et al, 1984) and CA release (Lim et al, 1992). Therefore, it was of interest to examine the effects of tacrine on Bay-K-8644-evoked CA secretion from the isolated perfused rat adrenal glands. Fig. 5 shows the inhibitory effect of 10^{-4} M tacrine on Bay-K-

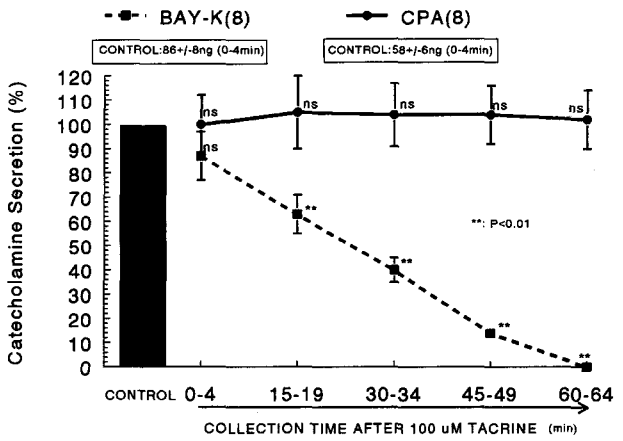

Fig. 5. Effects of tacrine on CA release evoked by Bay-K-8644 and cyclopiazonic acid from the rat adrenal glands. Bay-K-8644 (10^{-4} M) and cyclopiazonic acid (10^{-4} M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with of tacrine (10^{-4} M) for 60 min. Other legends are the same as in Fig. 1. BAY-K: Bay-K-8644, CPA: Cyclopiazonic acid. ns: Statistically insignificant.

8644-evoked CA secretory responses. In the absence of tacrine, Bay-K-8644 (10^{-5} M) given into the perfusion stream evoked CA secretion of 86 ± 10 ng (0~4 min) from 8 rat adrenal glands. However, in the presence of 10^{-4} M tacrine, Bay-K-8644-stimulated CA secretion was time-dependently inhibited to 87~0% of the corresponding control release.

Cyclopiazonic acid, a mycotoxin from *Aspergillus* and *Penicillium*, has been described as a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Georger & Riley, 1989; Seidler et al, 1989), and may be an extremely valuable pharmacological tool for investigating intracellular Ca^{2+} mobilization and ionic current regulated by intracellular calcium (Suzuki et al, 1992). In the present study, when cyclopiazonic acid (10^{-5} M) was given into the perfusion stream, the CA secreted from the gland amounted to 58 ± 6 ng for 4 min. As shown in Fig. 5, however, the pretreatment with tacrine failed to affect cyclopiazonic acid (10^{-5} M)-evoked CA secretion as compared to the control response (100%) from 8 adrenal glands.

Effect of physostigmine on CA secretion from the perfused rat adrenal glands evoked by ACh, high K^+, DMPP and McN-A-343

It has been reported that higher concentrations of tacrine and physostigmine inhibit the ACh-induced CA secretion by blocking nicotinic receptors, whereas lower concentrations of these drugs enhance such secretions due to their anticholinesterase actions (Sugawara et al, 1997). Therefore, it was of interest to examine the effect of physostigmine on CA secretion evoked by ACh, high K^+, DMPP and McN-A-343 from the isolated perfused rat adrenal glands.

As shown in Fig. 6, ACh (5.32 mM)-stimulated CA secretion before loading with physostigmine was 332 ± 36 ng (0~4 min) from 6 glands. However, in the presence of physostigmine (10^{-4} M) for 60 min, it was time-dependently

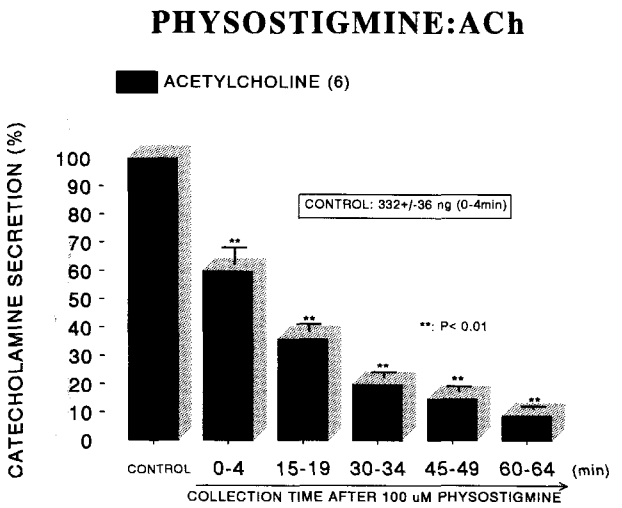

Fig. 6. Effect of physostigmine on CA release evoked by ACh. A single injection of ACh (5.32×10^{-3} M) was given at 15 min intervals during loading with physostigmine (10^{-4} M) for 60 min. Other legends are the same as in Fig. 1.

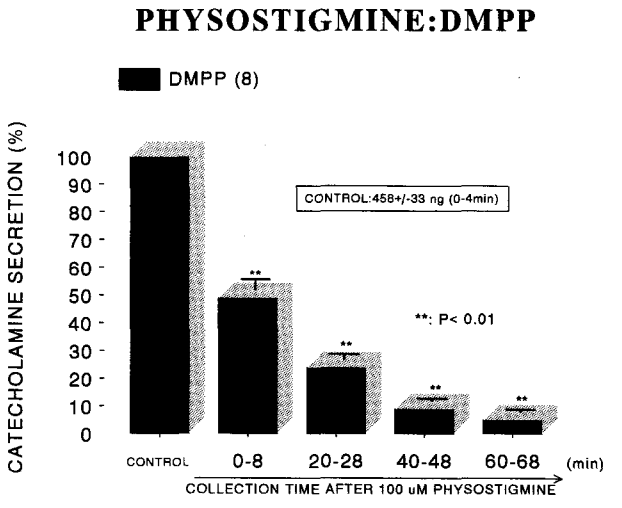

Fig. 8. Effect of physostigmine on CA release evoked by DMPP. CA secretion by a single injection of DPPP (10^{-4} M) was infused for 2 min at 20 min intervals during loading with physostigmine (10^{-4} M) for 60 min. Other legends are the same as in Fig. 1.

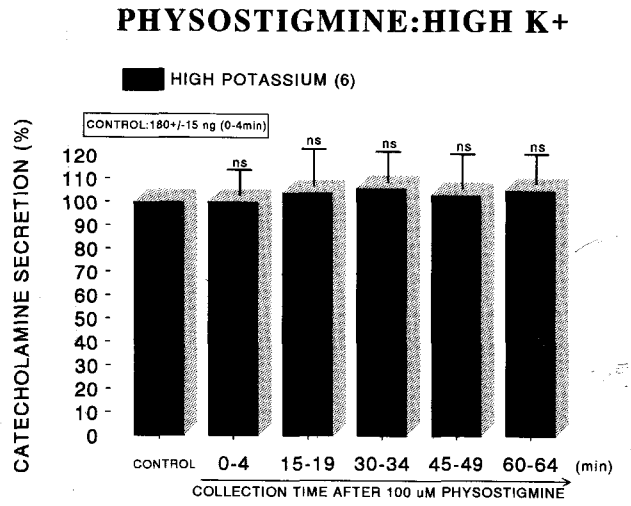

Fig. 7. Effect of physostigmine on CA release evoked by excess K^+. CA secretion by a single injection of K^+ (56 mM) was injected at 15 min intervals during loading with physostigmine (10^{-4} M) for 60 min. Other legends are the same as in Fig. 1. ns: Statistically insignificant.

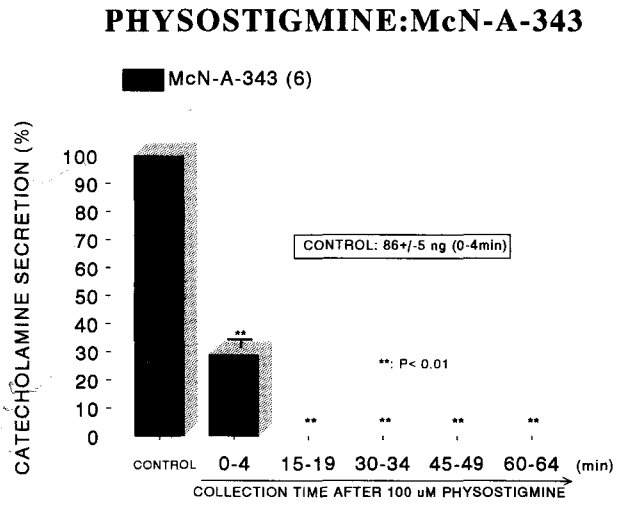

Fig. 9. Effect of physostigmine on CA release evoked by McN-A-343. Perfusion of McN-A-343 (10^{-4} M) for 4 min was induced at 15 min intervals during loading with physostigmine (10^{-4} M) for 60 min. Other legends are the same as in Fig. 1.

inhibited to 60~9% of the corresponding control secretory response (100%). In the presence of physostigmine (10^{-4} M), excess K^+ (56 mM)-evoked CA secretion was not changed as compared to the corresponding control secretion (180±15 ng) from 6 glands, as shown in Fig. 7. Before loading with physostigmine, DMPP (100 μM)-evoked CA secretory response was 458±33 ng (0~8 min). However, after perfusion with physostigmine (10^{-4} M)-containing Krebs solution it was significantly inhibited to 49~5% of the control secretion from 8 adrenal glands (Fig. 8). In 6 glands, McN-A-343 (100 μM)-evoked CA secretion before administration of physostigmine (10^{-4} M) was 86±5 ng (0~4 min), however, in the presence of physostigmine (10^{-4} M), McN-A-343-evoked CA secretion was strikingly reduced to 29~0% of the control release, as shown in Fig. 9.

DISCUSSION

The present results demonstrated that tacrine time- and concentration-dependently inhibits CA secretory responses evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors from the perfused model of the rat adrenal gland, but does fail to affect it by direct membrane-depolarization. Physostigmine also caused the similar inhibitory effects with that of tacrine. Furthermore,

tacrine time-dependently depressed CA secretion evoked by Bay-K-8644, but not by cyclopiazonic acid. It is highly likely that this inhibitory effect of tacrine may be exerted by blocking the calcium influx into the rat adrenomedullary chromaffin cells and without Ca^{2+} release from the cytoplasmic calcium store. It also seems likely that there is no difference in the mode of action between tacrine and physostigmine in rat adrenomedullary CA secretion.

In support of this idea, it has been found that both tacrine and physostigmine also inhibited CA induced by cholinergic agonists, nicotine and carbachol in the perfused adrenal glands of the guinea-pig (Sugawara et al, 1997). These results are in agreement with the present findings that tacine time- and concentration-dependently inhibited CA secretory responses evoked by DMPP and ACh from the perfused rat adrenal gland, but not by high K^+. Clarke & his colleagues (1994) have shown 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 nicotine-induced dopamine release, but also that induced by high K^+. On the other hand, it has been reported that tacrine and/or physostigmine inhibit voltage-dependent Na^+ channels in giant axons (Schauf & Sattin, 1987) and at neuromuscular junction (Elinder et al, 1989). It also inhibits K^+ channels in hippocampal neurons (Rogawski, 1987), snail neurons (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). It was also shown that in dispersed chromaffin cells, both CA secretion and inward current evoked by nicotine were inhibited by either tacrine or physostigmine with similar IC_{50} value (Sugawara et al, 1997). 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 of these two drugs in inhibiting the nicotine-induced secretory response and acetylcholinesterase activity are considerably different.

Tacrine and physostigmine failed to affect CA secretion evoked by high K^+ in guinea-pig adrenal chromaffin cells (Sugawara et al, 1998), in agreement with the result obtained in the present study. In support of this finding, physostigmine is reported not to affect the high K^+-evoked CA in cultured bovine adrenal chormaffin cells (Mizobe & Livett, 1982) and rat striatal synaptosomes (Clarke et al, 1994). However, tacrine (100 μM) has been shown to inhibit Ca^{2+} currents by more than 50% in rat nodose ganglia (Kelly et al, 1991). Tacrine (3 μM) is also known to decrease the high K^+-evoked dopamine secretion in rat striatal synaptosomes (Clarke et al, 1994). Based on these facts, it seems that there is difference in the sensitivities between species and tissues.

In contrast to the effect on high K^+-evoked CA secretion, the finding in the present investigation that tacrine and physostigmine time- and concentration-dependently inhibited CA secretory responses evoked by Bay-K-8644, which specifically activates an L-type, voltage-sensitive calcium channel, demonstrated that tacrine-induced inhibitory effect on CA release was due to the blockade of the voltage-sensitive calcium channels. In support of this idea, there are presently a plethora of literatures demonstrating that calcium influx through voltage-sensitive Ca^{2+} channels plays a key role in a physiological pathway for activation of adrenal CA secretion (Douglas, 1975; Kao & Schneider, 1986).

Moreover, it has been reported that tacrine and physostigmine inhibit the veratridine-induced CA secretion and depress the voltage-dependent Na^+ and Ca^{2+} currents in dose-dependent manners in guinea-pig adrenal chromaffin cells (Sugawara et al, 1998). They also showed that the inhibitory action of tacrine was much more potent than that of physostigmine, and both drugs were more effective in decreasing Na^+ currents than Ca^{2+} currents. However, in the present experiments, the inhibitory effects of physostigmine were found to be more potent than that of tacrine in CA secretory effects evoked by cholinergic (nicotinic and muscarinic) stimulation. The difference in the susceptibility to the inhibitory activity of tacrine in CA secretion between responses to Bay-K-8644 and high K^+ might be due to the difference in the subtypes of Ca^{2+} channels involved. In fact, adrenal chromaffin cells have been reported to have only the high voltage-activated type of Ca^{2+} channels, which are classified as N-, L-, P-, and Q-type channels in ox (Lopez et al, 1994), rat (Gandia et al, 1995) and pig (Kitamura et al, 1997) by use of their selective blockers. However, definite subtypes of Ca^{2+} channels in the rat adrenal chromaffin cells have not been established yet. In the present experiments, it is felt that inhibitory effect of tacrine on voltage-dependent Ca^{2+} channel was responsible for the difference in the actions on secretory responses induced by high K^+ and Bay-K-8644, because Bay-K-8644 has been known to potentiate the release of CA by increasing Ca^{2+} influx through L-type Ca^{2+} channels in cultured bovine chromaffin cells (Garcia et al, 1984).

The results in the present investigation that tacrine as well as physostigmine inhibited CA secretion evoked by McN-A-343, a selective muscarinic M_1-receptor agonist, suggested strongly that muscarinic M_1-receptor was involved in the regulation of the CA secretory responses in the rat adrenal medulla. In support of this hypothesis, it has been shown that muscarinic stimulation generates a depolarizing signal which triggers the firing of action potentials, resulting in the increased CA release in the rat chromaffin cells (Akaike et al, 1990; Lim & Hwang, 1991). These observations are in line with the previous reports (Ladona et al, 1987; Uceda et al, 1992) showing that Bay-K-8644 almost tripled the peak secretory response to muscarine in the perfused cat adrenal glands. In the present experiments, both tacrine and physostigmine also depressed greatly CA secretion induced by Bay-K-8644. This finding that tacrine inhibited Bay-K-8644-evoked CA secretion suggests that the tacrine inhibits directly the voltage-dependent Ca^{2+} channels through the blockade of Ca^{2+} channels, just like Ca^{2+} channel blockers (Cena et al, 1983), which have direct actions on voltage-dependent Ca^{2+} channels. In the bovine chromaffin cells, stimulation of nicotinic, but not muscarinic ACh receptors, is known to cause CA secretion by increasing Ca^{2+} influx largely through voltage-dependent Ca^{2+} channels (Oka et al, 1979; Burgoyne, 1984).

In the present work, tacrine failed to affect the CA secretion evoked by cyclopiazonic acid. Cyclopiazonic acid is known to be a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Geoger & Riley, 1989; Seidler et al, 1989) and a valuable pharmacological tool for investigating intracellular Ca^{2+} mobilization and

ionic currents regulated by intracellular Ca^{2+} (Suzuki et al, 1992). Therefore, it is likely that the inhibitory effect of tacrine on CA secretion evoked by cholinergic stimulation may not be associated with the mobilization of intracellular Ca^{2+} in the chromaffin cells. Previously, 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; Flynn & Mash, 1989; Nielsen et al, 1989), and intracellular Ca^{2+}-dependent K^+ channels, probably of the small-conductance type (SK), seem to be involved in the modulation of muscarinic-evoked CA release responses in cat adrenal chromaffin cells (Uceda et al, 1992). However, in the present study, the fact that McN-A-343-evoked CA secretion was depressed by pretreatment with tacrine suggested relevance with these earlier results. Whether tacrine has the blocking effect of muscarinic M_1-recptors in CA secretion from the adrenal gland remains to be resolved in future.

On the other hand, in contrast to the present results, various mechanisms of action of tacrine on transmitter release have been reported. For example, tacrine augments neuromuscular transmission due to the inhibition of acetylcholinesterase (Braga et al, 1991), stimulates the spontaneous secretion of large quanta of ACh at motor nerve terminals (Thesleff et al, 1990), and displaces norepinephrine from intraneuronal transmitter stores of sympathetically innervated tissues (Fabiani et al, 1992). Tacrine has been found to enhance ACh-evoked CA secretion with lower concentrations and to inhibit it with higher concentrations in perfused adrenal glands of the guinea pig (Sugawara et al, 1997). This finding suggests that the enhancing effect of tacrine is attributed to its anti-acetylcholinesterase action, as suggested by Heilbronn (1961). Moreover, tacrine has been demonstrated to induce the increased plasma levels of norepinephrine and epinephrine, suggesting a rise in sympathetic tone following tacrine administration (Allal et al, 1998). Tacrine also enhances monoamine neurotransmission in the rat striatum, probably via an interaction with both muscarinic and nicotinic heteroreceptors (Warpman et al, 1996). Recently, it has been reported that both intravenous and intracerebroventricular tacrine stimulates central muscarinic cholinoceptors to increase blood pressure in normotensive rats and that increases of plasma CA and vasopressin are both involved in this response (Savci et al, 1998). Therefore, further study on the binding of nicotinic and muscarinic receptors responsible for tacrine-stimulated CA secretion remains to be carried out.

In conclusion, the present results collectively demonstrate that tacrine greatly inhibits CA secretion evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors from the perfused rat adrenal gland, but does fail to affect that by direct membrane-depolarization. This inhibitory effect of tacrine may be exerted by blocking the calcium influx into the rat adrenomedullary chromaffin cells without Ca^{2+} release from the cytoplasmic calcium store, of which is relevant to the cholinergic blockade. Finally, it also seems that there exists a similarity in the mode of action between tacrine and physostigmine in rat adrenomedullary CA secretion.

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