

Positive Feedback Modulation of Acetylcholine Release from Isolated Rat Superior Cervical Ganglion¹

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ABSTRACT

The effects of selective nicotinic acetylcholine (ACh) receptor (nAChR) agonists and antagonists on the stimulation-evoked release of [³H]ACh were studied in rat isolated superior cervical ganglion loaded with [³H]choline and superfused in a 2-ml chamber. Nicotine and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), but not cytisine, increased the stimulation (2 Hz)-evoked release of [³H]ACh in a concentration-dependent manner. The rank order of potency to increase stimulation-evoked release for the nAChR agonists (nicotine > DMPP >> cytisine) suggests that the $\beta 4$ subunit of nAChRs is not involved in the release. The finding that α -bungarotoxin was effective in preventing the effect of DMPP and itself significantly reduced the release indicates that the $\alpha 7$ subunit is located presynaptically and may be involved in the positive feedback modulation. Hexamethonium inhibited the effect of DMPP with an apparent dissociation constant (K_d) of $11.5 \pm 1.5 \mu\text{M}$. Hexamethonium and other nAChR antagonists, *i.e.*, (+)-tubocurarine

(100 μM), mecamylamine (3 μM), dihydro- β -erythroidine (3 μM), pancuronium (10 μM) and α -bungarotoxin (2 μM), also decreased the stimulation-evoked release of [³H]ACh. The effect of hexamethonium was independent of stimulation frequency (2, 10 and 30 Hz) applied. Atropine enhanced the stimulation-evoked release of ACh, indicating that there is negative feedback modulation of ACh release associated with neuronal activity. In contrast, when the nicotinic positive feedback was prevented by hexamethonium, atropine failed to enhance the release. These findings indicate that muscarinic receptor-mediated inhibition of ACh release functions in cases in which the release is enhanced by ACh *via* stimulation of presynaptic nAChRs. A similar interaction was found between A₁ receptor-mediated reduction and nAChR-mediated positive feedback modulation of [³H]ACh release. The results suggest the presence of positive feedback modulation of ACh release *via* presynaptic nAChRs in rat superior cervical ganglion.

The pharmacological subclassification of nAChRs was first described by Paton and Zaimis (1952). They showed that antagonists are able to select between nicotinic synapses in autonomic ganglia and in muscle, and they thus provided indirect pharmacological evidence that nAChRs are heterogeneous. Molecular cloning and physiological techniques have subsequently revealed that neuronal nAChRs are clearly distinct from muscle nAChRs and are themselves diverse (Deneris *et al.*, 1991; Sargent, 1993). nAChRs are present on autonomic neurons and adrenal chromaffin cells in the peripheral nervous system and on many neurons in the central nervous system. Strong neurochemical and pharmacological evidence that nAChRs, in addition to their postsynaptic localization, are located presynaptically and are involved in the modulation of transmitter release has been obtained. nAChR stimulation enhances the release of ACh from the cortex (Rowell and Winkler, 1984; Nordberg *et al.*,

1989) and neuromuscular junction (Wessler *et al.*, 1986, 1987; Gibb and Marshall, 1986; Somogyi and Vizi, 1987; Vizi *et al.*, 1987, 1995; Vizi and Somogyi, 1989), indicating positive feedback modulation of ACh release. It has also been shown that stimulation of nAChRs leads to release of different catecholamines (*e.g.*, norepinephrine and dopamine) in the peripheral nervous system (Todorov *et al.*, 1991) and central nervous system (Westfall, 1974; Rapier *et al.*, 1988; Sándor *et al.*, 1991; Grady *et al.*, 1992; Hársing *et al.*, 1992; Vizi *et al.*, 1995; Sacaan *et al.*, 1995).

In addition, there is agreement that cholinergic axon terminals are endowed with muscarinic autoreceptors that serve to modulate the release of ACh *via* ACh stimulation of muscarinic receptors (Fosbraey and Johnson, 1980; Vizi *et al.*, 1984, 1991; Somogyi and Vizi, 1987; Somogyi *et al.*, 1987; Vizi and Somogyi, 1989; Milusheva *et al.*, 1994), for example, those located in the SCG (Capuzzo *et al.*, 1988, 1989). The present study describes the pharmacological properties of nAChRs located in isolated SCG at the presynaptic level, *i.e.*, on axon terminals, and the role of A₁ and muscarinic receptors in the modulation of [³H]ACh release.

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ABBREVIATIONS: ACh, acetylcholine; DMPP, 1,1-dimethyl-4-phenylpiperazinium iodide; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; nAChR, nicotinic acetylcholine receptor; S₁, first stimulation; S₂, second stimulation; SCG, superior cervical ganglion.

Materials and Methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care Committee of the Institute. All procedures conformed to the Guiding Principles of the Medical Ethics Committee.

Preparation of the cervical ganglion. Rats (180–220 g) of either gender were anesthetized with ether and decapitated. Both ganglia, together with the preganglionic sympathetic nerve and the postganglionic (internal carotid) nerve, were isolated, desheathed and incubated in 1 ml of Krebs solution (113 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 25.0 mM NaHCO₃, 11.5 mM glucose), containing 0.13 μ M [*methyl*-³H]choline chloride (specific activity, 75 Ci/mmol; Amersham), for 40 min. The medium was bubbled continuously with 95% O₂/5% CO₂ at 37°C. After incubation, the preparations were transferred to an organ bath of 2-ml volume and superfused continuously with Krebs solution at a rate of 0.6 ml/min. The Krebs solution contained hemicholinium-3 (10 μ M) to prevent the reuptake of [³H]choline derived from the hydrolysis of [³H]ACh. After a 60-min period to wash out the excess radioactivity and to allow equilibration of tissues, 5-min superfusate samples were collected with an automatic fraction collector and assayed for [³H]ACh. Electrical field stimulation was applied at two times (S₁ and S₂, separated by a 35-min interval), through a pair of platinum electrodes, by means of an Eltron (Budapest, Hungary) stimulator [2 Hz for 2.5 min, 10 Hz for 0.5 min and 30 Hz for 10 sec; *i.e.*, 300 shocks were delivered (voltage drop, >10 V/cm; pulse width, 0.5 msec)]. Ganglia dissected from one side were used for control experiments, and the corresponding contralateral ganglia were used for experiments in which atropine or nAChR antagonists were added 20 min before S₂ and kept in the solution throughout the experiments. nAChR agonists (DMPP, cytosine and nicotine) were added 10 sec, 2 min or 10 min before S₂ as indicated. When the effects of nAChR agonists and antagonists on resting release were studied, S₂ was not applied.

In some experiments, the apparent dissociation constant (K_d) of nAChR antagonists was also calculated, using the equation $K_d = [a]/(DR - 1)$, where a is the concentration of antagonist and DR is the dose ratio between the equipotent concentrations of DMPP in the presence and absence of antagonist.

[³H]ACh assay. Radioactivity released from the preparations was determined by adding a 0.5-ml aliquot of the superfusate sample to 2 ml of liquid scintillation fluid (Packard Ultima Gold) and counting the sample in a Packard 544 liquid scintillation counter. To determine residual radioactivity, the tissues were blotted on filter paper and weighed, and the radioactivity was extracted with 10% trichloroacetic acid. The counts were converted to absolute activity by using an external standard. Because the tritium content of the tissue was determined at the end of each experiment (Vizi *et al.*, 1984) and the efflux of radioactivity was measured throughout the experiments, it was possible to calculate the fractional release that occurred during each 5-min collection period. The stimulation-evoked increase of radioactivity above the resting level was also expressed as a percentage of the total ³H present in the tissue at the onset of each stimulation. An IBM desk computer was used for calculation. The tissue content of ACh was also estimated as described by Vizi *et al.* (1985).

Analysis. The results were evaluated by Student's *t* test for paired or unpaired observations as appropriate. *P* values of <0.05 were considered to be statistically significant. In some experiments one-way analysis of variance was used, followed by the Tukey-Kramer multiple-comparison test. All data were expressed as means \pm S.E.M.

Drugs. The following drugs were used: DMPP, hexamethonium bromide, atropine sulfate, (+)-tubocurarine chloride, physostigmine sulfate, (–)-nicotine hydrogen tartrate and hemicholinium-3 were purchased from Sigma Chemical Co. (St. Louis, MO); cytosine, mecamlamine hydrochloride, dihydro- β -erythroidine, α -bungaro-

toxin and DPCPX were purchased from RBI; and pancuronium was received from Organon (The Netherlands).

Results

Content and release of [³H]ACh. The ACh content of the ganglion was 22.4 ± 1.2 nmol/g ($n = 4$). After the tissue had been loaded with [³H]choline, the radioactivity content of tissue was $441,390 \pm 15,930$ Bq/g ($n = 24$). In six experiments, cholinesterase activity was inhibited with physostigmine (2 μ M), and [³H]choline and [³H]ACh were separated by the method of Vizi *et al.* (1984). Under these conditions, $93.2 \pm 3.8\%$ of the radioactivity released by electrical field stimulation (2 Hz, 300 shocks) was [³H]ACh. Under resting conditions, this value was $52.4 \pm 2.8\%$. In additional experiments physostigmine was not used, to avoid the synthesis and storage of surplus ACh (Birks and MacIntosh, 1961; Collier and Katz, 1970; Collier *et al.*, 1983).

The spontaneous (resting) release of radioactivity collected in 5 min was $13,766 \pm 964$ Bq/g, $2.9 \pm 0.2\%$ of the radioactivity content of the tissue. Electrical field stimulation (2 Hz, 300 shocks) released [³H]ACh. The amount of released radioactivity was $9.87 \pm 0.93\%$ released by S₁ and $7.42 \pm 0.82\%$ released by S₂ (fig. 1). The S₂/S₁ ratio was 0.73 ± 0.08 ($n = 8$).

Effect of nAChR agonists and antagonists. Although the resting release was not affected (data not shown), the stimulation-evoked release of [³H]ACh was enhanced by the nAChR agonists in a concentration-dependent manner. The nAChR agonists added 10 min before S₂ increased the release of [³H]ACh, as indicated by the increase of the S₂/S₁ ratio. In five experiments, we estimated the relative efficacy of nicotinic agonists (nicotine, cytosine and DMPP) by comparing the amount of [³H]ACh (fractional release) released by various concentrations of these agonists (up to 100 μ M) and the amount of [³H]ACh released by 50 μ M DMPP (fig. 2). The rank order of agonist efficacy was nicotine > DMPP > cytosine. The equieffective concentrations of nicotine, DMPP and cytosine were 1.6, 30.5 and >100 μ M, respectively. The effect of DMPP (100 μ M) was not significantly different if it was present for 10 sec or 2 min, compared with 10 min, before S₂: the S₂/S₁ values were 1.12 ± 0.06 , 1.16 ± 0.08 and $1.01 \pm$

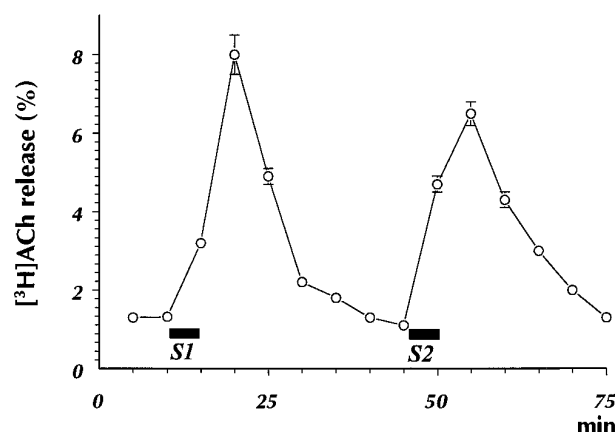


Fig. 1. Effect of field stimulation (S₁ and S₂) on the fractional release of [³H]ACh from the rat SCG. Collection periods (5 min) and stimulation (S₁ and S₂; 2 Hz, 300 shocks) are indicated. The release at rest in a 5-min collection period was $1.63 \pm 0.06\%$ of total activity (fractional release). The release in response to stimulation was $9.87 \pm 0.93\%$ for S₁ and $7.42 \pm 0.82\%$ for S₂. The S₂/S₁ ratio was 0.73 ± 0.08 ($n = 8$).

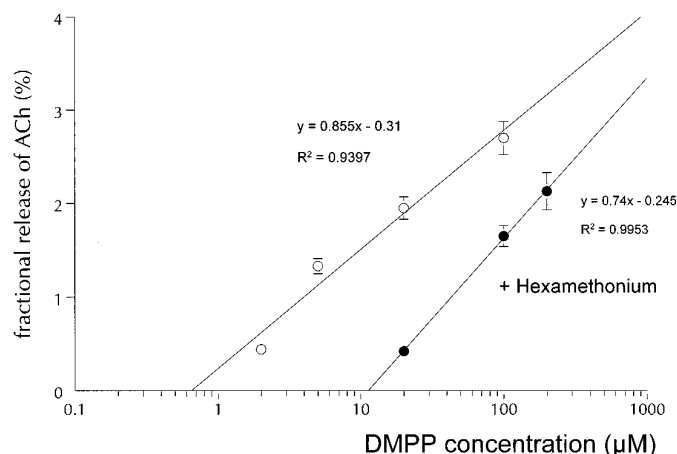


Fig. 2. Effect of DMPP and a nicotinic antagonist (hexamethonium) on the stimulation-evoked release of [^3H]ACh. Ganglia were stimulated two times (S_1 and S_2 ; 2 Hz, 300 shocks), with a 30-min interval. Hexamethonium at 100 μM and DMPP at different concentrations were added to the perfusion Krebs solution 20 min and 10 min before S_2 and were kept in the solution throughout the experiments. Note that DMPP potentiated the release in a concentration-dependent manner and hexamethonium shifted the dose-response curve to the right. Values are the mean \pm S.D. of six tests.

0.06, respectively ($P > .025$). The nicotinic response to DMPP was blocked by various classical antagonists [mecamylamine, (+)-tubocurarine, hexamethonium and α -bungarotoxin]. Dose-response curves for antagonists were obtained with a test administration of 50 μM DMPP. The IC_{50} values are shown in table 1. Except for hexamethonium, no attempt was made to study the mode of action of the antagonists. Hexamethonium (100 μM) blocked the facilitatory effect of DMPP on the release of [^3H]ACh evoked by stimulation and shifted the dose-response curve to the right (fig. 2). The apparent K_d was 11.5 ± 1.5 μM (fig. 2).

The next series of experiments determined whether endogenous ligand, ACh released by axonal activity into the synaptic gap, affected the release of [^3H]ACh. For this purpose, nAChR and muscarinic ACh receptor antagonists were used (table 2). Hexamethonium administered alone significantly reduced the release evoked by stimulation (tables 2 and 3). Similarly, other nAChR antagonists, *i.e.*, (+)-tubocurarine (100 μM), mecamylamine (3 μM), dihydro- β -erythroidine (3 μM), pancuronium (10 μM) and α -bungarotoxin (2 μM), significantly decreased the stimulation-evoked release of [^3H]ACh from the SCG (table 2). This finding indicates that there is positive feedback modulation of ACh release. The muscarinic receptor antagonist atropine, added before S_2 ,

TABLE 1
 IC_{50} values of various antagonists for nAChRs located on axon terminals

Different concentrations of the antagonists were tested on the response to 50 μM DMPP. Values are mean \pm S.E.M. ($n = 4-6$).

Antagonists	IC_{50} μM
α -Bungarotoxin	2.5 ± 0.3
Mecamylamine	3.5 ± 0.2
Dihydro- β -erythroidine	6.0 ± 0.3
Pancuronium	14.0 ± 0.9
Hexamethonium	20.0 ± 1.8
(+)-Tubocurarine	85.0 ± 14.7

TABLE 2

Effect of DMPP, (–)-nicotine and nAChR antagonists on the evoked release of [^3H]ACh (stimulation rate, 2 Hz, 300 shocks)

Drugs	Effect on [^3H]ACh Release (S_2/S_1) ^a	
	Control	Drug Present
DMPP, 100 μM	0.74 ± 0.02	$1.01 \pm 0.06^*$
(–)-Nicotine, 1 μM	0.70 ± 0.04	0.88 ± 0.07
(–)-Nicotine, 10 μM	0.74 ± 0.01	$1.08 \pm 0.05^{**}$
(–)-Nicotine, 50 μM	0.65 ± 0.06	$1.21 \pm 0.07^{**}$
Cytisine, 100 μM	0.71 ± 0.03	0.72 ± 0.06
Hexamethonium, 100 μM , + DMPP, 100 μM	0.76 ± 0.05	$0.56 \pm 0.04^{**\dagger}$
Hexamethonium, 100 μM	0.73 ± 0.03	$0.28 \pm 0.09^{**}$
(+)-Tubocurarine, 100 μM	0.71 ± 0.06	$0.34 \pm 0.05^*$
(+)-Tubocurarine, 100 μM , + DMPP, 20 μM	0.72 ± 0.03	$0.39 \pm 0.05^{**\dagger}$
Mecamylamine, 3 μM	0.66 ± 0.04	$0.48 \pm 0.03^*$
Dihydro- β -erythroidine, 3 μM	0.65 ± 0.02	$0.45 \pm 0.07^*$
Pancuronium, 10 μM	0.72 ± 0.02	$0.43 \pm 0.08^*$
α -Bungarotoxin, 2 μM	0.66 ± 0.06	$0.35 \pm 0.07^{**}$
α -Bungarotoxin, 2 μM , + DMPP, 10 μM	0.79 ± 0.08	$0.51 \pm 0.04^{**\dagger}$
Atropine, 0.3 μM	0.76 ± 0.08	$1.28 \pm 0.17^{**}$
Atropine, 0.3 μM , + hexamethonium, 100 μM	0.73 ± 0.04	$0.38 \pm 0.05^{**}$

^a The results are expressed as S_2/S_1 ratios. The values are means \pm S.E.M. The drugs (antagonists) were administered 20 min before S_2 and kept in the superfusate throughout the experiment. nAChR agonists (DMPP and nicotine) were added 10 min before S_2 . Contralateral ganglia dissected from the same animal were used for control experiments.

* Significant ($P < .05$) difference from the corresponding control value.

** Significant ($P < .01$) difference from the corresponding control value (Student's *t* test).

† Significance ($P < .05$) calculated between release of [^3H]ACh in the presence of DMPP and DMPP plus antagonists [hexamethonium, (+)-tubocurarine or α -bungarotoxin] (analysis of variance followed by Tukey-Kramer multiple-comparison test). Note that, in the presence of hexamethonium, atropine failed to increase the release of ACh ($P < .01$). The number of experiments is five in each group.

significantly enhanced the stimulation-evoked (2 Hz, 300 shocks) release of ACh; the S_2/S_1 ratio was increased from 0.76 ± 0.08 to 1.28 ± 0.17 ($n = 5$) ($P < .01$). When the positive feedback modulation *via* nAChRs was prevented by hexamethonium, atropine failed to increase the amount of ACh released by field stimulation (table 2).

Because evidence has been obtained that ATP is released with ACh from the SCG, and its breakdown product adenosine is able to reduce the release of ACh *via* stimulation of A_1 receptors (E. S. Vizi and S. D. Liang, unpublished data), the A_1 receptor antagonist DPCPX was tested (table 3) at a different frequency of stimulation. DPCPX enhanced and hexamethonium reduced the stimulation-evoked release of ACh at each stimulation frequency applied. In the presence of hexamethonium, DPCPX was not able to enhance the release of ACh. The release in the presence of both drugs was similar to that obtained with hexamethonium alone (table 3).

Discussion

In sympathetic ganglia, ACh is the major neurotransmitter (Feldberg and Gaddum, 1934; Collier and Katz, 1970; Dawes and Vizi, 1973; Collier *et al.*, 1983; Briggs *et al.*, 1985; Somogyi and De Groat, 1993). The release of ACh in the resting state and in response to low-frequency stimulation from nerve terminals in the SCG was estimated by measuring ^3H in samples in the absence of cholinesterase inhibitors. The presence of hemicholinium, which blocks choline uptake, en-

TABLE 3

Effect of hexamethonium on the release of [^3H]ACh evoked by different-frequency electrical stimulation under conditions in which the A receptor-mediated inhibition by endogenous adenosine was abolished by DPCPX

Frequency Hz	Fractional Release of ACh with Stimulation ^a			
	Control	Hexamethonium (100 μM)	DPCPX (10 nM)	DPCPX (10 nM) + Hexamethonium (100 μM)
			%	
2	7.49 \pm 1.08	3.41 \pm 0.78*	10.83 \pm 0.90	3.98 \pm 0.63*†
10	9.24 \pm 0.87	4.34 \pm 0.33*	13.36 \pm 0.94*	4.94 \pm 0.58*†
30	10.02 \pm 0.63	5.61 \pm 0.78*	14.11 \pm 0.87*	5.42 \pm 1.13*†

^a The same number of shocks were applied (300). The total amount of radioactivity released by S_2 was taken into account and was expressed as percent of radioactivity present in the tissue.

* Significance ($P < .05$), in comparison with control values.

† Significance ($P < .001$, analysis of variance followed by Tukey-Kramer multiple-comparison test) for DPCPX vs. DPCPX plus hexamethonium. Note that hexamethonium reduced the release of ACh even when the release was enhanced by the presence of DPCPX. The number of experiments is four in each group, except the control group ($n = 6$).

sured that the radioactivity (^3H]choline) derived from hydrolysis of ACh (Collier and Katz, 1970; Somogyi and De Groat, 1993) could be measured in the superfusate after electrical stimulation. This was confirmed in our experiments by measuring the release of ^3H]choline and ^3H]ACh under conditions in which cholinesterase was inhibited by physostigmine (2 μM) (Vizi *et al.*, 1984).

Electrical stimulation released ^3H]ACh (fig. 1). The pharmacology of presynaptic nAChRs has been assessed by measuring the effects of nAChR agonists and antagonists on ^3H]ACh release evoked by neuronal stimulation. DMPP and nicotine potentiated the release in a concentration-dependent manner, and cytisine failed to affect it (table 2; fig. 2). DMPP and nicotine had no effect on the resting release. The ability of DMPP and nicotine to increase the stimulation-evoked release of ACh from isolated SCG suggests that DMPP and nicotine can act at a presynaptic site. The rank order of nAChR agonists, *i.e.*, nicotine $>$ DMPP \gg cytisine, suggests that the $\beta 4$ subunit of nAChRs is not involved in the release. Binding studies (Whiting *et al.*, 1991) suggest that cytisine is a more potent agonist than nicotine and its potency depends on the subunit composition of the nAChR. Cytisine can distinguish between receptors containing $\beta 4$, which are more sensitive to cytisine than to nicotine, and receptors containing $\beta 2$, which are much less sensitive (Lutjens and Patrick, 1991; Sargent, 1993).

The findings that nAChR antagonists such as hexamethonium, (+)-tubocurarine, mecamylamine, dihydro- β -erythroidine, pancuronium and α -bungarotoxin significantly reduced the stimulation-evoked release of ACh indicate that the release is under the tonic influence of ACh. However, the rank order of potency of these antagonists [α -bungarotoxin $>$ mecamylamine $>$ dihydro- β -erythroidine $>$ pancuronium $>$ hexamethonium $>$ (+)-tubocurarine] was different from that for their ganglion-blocking properties [mecamylamine $>$ (+)-tubocurarine $>$ pancuronium $>$ hexamethonium \gg α -bungarotoxin] (Bowman and Webb, 1972; Dunn and Karczmar, 1980), suggesting that there are different functioning nAChR subtypes in rat SCG. Although both pre- and postsynaptic nAChRs in the ganglion are neuronal, the results with antagonists suggest that the presynaptic receptors differ pharmacologically from the postsynaptic receptors. Our finding that α -bungarotoxin was effective in preventing the effect of DMPP and itself significantly reduced the release indicates that the $\alpha 7$ subunit may be involved in increasing the stimulation-evoked release of ^3H]ACh. This is supported by the

findings that the $\alpha 3$, $\alpha 5$, $\alpha 7$, $\beta 2$ and $\beta 4$ transcripts are present in the SCG of rats (Mandelzys *et al.*, 1994; de Koninck and Cooper, 1995) and heterooligomeric receptors containing both $\alpha 7$ and $\alpha 3$ subunits are found in chick ciliary ganglion neurons (Listerud *et al.*, 1991). Therefore, it seems likely that the $\alpha 7$ subunit is localized presynaptically and is involved in the potentiation of ACh release associated with neuronal activity. The function of the $\alpha 7$ subunit is poorly understood; however, recent studies indicate that this receptor promotes Ca^{++} influx (Séguéla *et al.*, 1993; Castro and Albuquerque, 1995) and may affect various Ca^{++} -mediated processes, such as transmitter release. Previous work showed that α -bungarotoxin failed to block ganglionic transmission in rat SCG (Brown and Fumagilli, 1977; Dunn and Karczmar, 1980) but inhibited the membrane depolarization induced by iontophoretic application of ACh or carbachol to the surface of ganglion cells (Dunn and Karczmar, 1980), an effect that was also inhibited by (+)-tubocurarine.

Our results demonstrate that nAChRs that modulate ACh release associated with axonal activity do not undergo agonist-induced desensitization, a phenomenon characteristic of nAChRs. At least, the effectiveness of DMPP to potentiate the stimulation-evoked release of ACh was not significantly reduced when the exposure time was increased from 10 sec to 10 min.

The muscarinic receptor antagonist atropine enhanced the release of ACh, indicating that, in addition to nicotinic (positive) feedback modulation, muscarinic receptor-mediated negative feedback modulation occurs in the SCG (Capuzzo *et al.*, 1988, 1989). It seems likely that ACh, being the endogenous ligand for both muscarinic ACh receptors and nAChRs, stimulated both receptor types located presynaptically, with opposing effects. The effect of ACh to enhance its own release is counteracted by its effect on muscarinic receptors. A similar interaction was observed between A_1 receptor-mediated inhibition and nAChR-mediated positive feedback modulation of ACh release. Inhibition of adenosine receptors by the selective A_1 receptor antagonist DPCPX resulted in an increase of ACh release. This finding indicates that the release of ACh is also controlled by endogenous adenosine. The finding that there was no significant difference between the effect of DPCPX and that of hexamethonium on ACh release evoked by a different stimulation frequency (table 3) indicates that the biophase concentrations of ACh and adenosine and their modulatory presynaptic effects became saturated even at the lower (2 Hz) stimulation rate. In the absence of

positive feedback modulation, *i.e.*, in the presence of a presynaptically active antinicotinic drug, DPCPX failed to enhance the release, indicating that A₁ receptor-mediated inhibition is operative only when the release is enhanced by positive feedback modulation. In addition, it was reported that the release of ACh from isolated SCG of rabbits was reduced through stimulation of presynaptic α adrenoceptors (Christ and Nishi, 1971; Dawes and Vizi, 1973). These pharmacological data suggest that ganglionic transmission is presynaptically controlled by different endogenous ligands (including adenosine, norepinephrine and ACh) through different receptors.

Koelle (1961) proposed that ACh released from presynaptic terminals by a nerve impulse may have a dual role in synaptic transmission, *i.e.*, an action at the presynaptic site, affecting the release of additional quanta of ACh, and a transmitter action at the postsynaptic site. The main criticism (Collier and Katz, 1970) of Koelle's hypothesis was that, in the presence of physostigmine, the nerve terminals in the ganglion synthesize and store ACh in excess of their normal transmitter depot (Birks and MacIntosh, 1961) and this "surplus" ACh was probably the source of the ACh released by carbachol. The method developed here has allowed us to test the presynaptic effect of ACh under conditions in which cholinesterase was not inhibited. In this study, the lack of effect of nAChR agonists on spontaneous efflux of [³H]ACh does not support the original hypothesis of Koelle (1961) but the finding that stimulation-evoked release of ACh is enhanced by positive feedback modulation is compatible with the notion of presynaptic effects of ACh, as first suggested by Koelle (1961).

It remains to be determined whether a homooligomer of $\alpha 7$ or its combination with other subunits may constitute a preganglionic nAChR subtype that is sensitive to α -bungarotoxin. The finding that, in addition to α -bungarotoxin, other nAChR antagonists influenced the release suggests that a combination of $\alpha 7$ with other subunits form these receptors. However, additional experiments are needed to establish whether this is the case.

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