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POSSIBLE MUSCARINIC REGULATION OF CATECHOLAMINE SECRETION MEDIATED BY CYCLIC GMP IN ISOLATED BOVINE ADRENAL CHROMAFFIN CELLS

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Abstract—Catecholamine secretion and cyclic GMP levels were measured in chromaffin cells isolated from bovine adrenal medulla. Acetylcholine (ACh) and nicotine, but not muscarine, induced 8- to 10-fold increases in catecholamine secretion, with respective ED_{50} values of 10 and 2 μM. Cyclic GMP levels were also increased from 3- to 5-fold in the presence of ACh, and this stimulation was mimicked by muscarine but not by nicotine. Half-maximum stimulations of cyclic GMP levels with ACh and muscarine were observed at 0.1 and 0.3 μM respectively. The order of potency of various cholinergic drugs for cyclic GMP stimulation was as follows: ACh > oxotremorine > methacholine > muscarine > carbamylcholine > furthretonium > arecholine > bethanechol. Pilocarpine, McN-A-343, and AHR-602 were inactive at concentrations between 10^{-8} and 10^{-3} M. Isobutylmethylxanthine (1 mM), a specific phosphodiesterase inhibitor, caused a 7-fold increase in cyclic GMP and potentiated 3-fold the stimulation of cyclic GMP by ACh. The nicotine-induced catecholamine secretion was inhibited 19 and 33 per cent by the co-stimulation of the muscarinic receptor with 0.2 and 0.5 μM ACh, respectively. Isobutylmethylxanthine (1 mM) also caused a 44 per cent inhibition of nicotine-induced catecholamine secretion, and its effect was additive to that of ACh. Atropine (0.1 μM) selectively abolished the inhibition caused by ACh. Similar inhibitions were also obtained in the presence of exogenous dibutyryl cyclic GMP or 8-bromo cyclic GMP. These data indicate that the nicotinic stimulation of catecholamine secretion from bovine adrenal chromaffin cells may be regulated by cyclic GMP via the stimulation of a muscarinic receptor.

The adrenal medulla has been used extensively to study the mechanism of "stimulus–secretion coupling" first described by Douglas [1]. In this system, acetylcholine (ACh)†, the known transynaptic mediator of catecholamine (CA) secretion, binds to a specific receptor located on the chromaffin cell thereby inducing changes in membrane permeability to ions [2–6] and, in the presence of calcium, the release of CA by exocytosis [7, 8]. CA secretion from the adrenal medulla can also be regulated in a way similar to that of noradrenaline output from adrenergic nerve fibers [9]. Modulation of CA secretion from nerve fibers has been shown to occur via the stimulation of various receptors located on the adrenergic nerve terminals, including the α-adrenergic, prostaglandin, and muscarinic receptors [9]. All of these receptors, with the exception of the muscarinic type, have also been shown to inhibit the release of CA from the adrenal medulla [10–12]. In many animal species, however, stimulation of an adrenal muscarinic receptor produces a stimulation of CA release instead of an inhibition of CA secretion, as found in noradrenergic nerve fibers [9]. Furthermore, in the bovine adrenal gland, although the presence of a muscarinic receptor has already been demonstrated [13], the secretory effect of ACh had been found to be related exclusively to the stimulation of a nicotinic receptor [7, 14, 15].

Using a preparation of bovine adrenal chromaffin cells, we have recently reported that ACh stimulates cyclic GMP accumulation, and a muscarinic receptor was postulated to be responsible for this increase [16]. The present study further examines the type of receptor involved in the cyclic GMP response to ACh and emphasizes a possible regulatory function for cyclic GMP in CA secretion via the specific stimulation of a muscarinic receptor.

METHODS

Cell preparation. Bovine adrenal glands were rapidly removed from the animals after exsanguination and decapitation in a local slaughterhouse. The glands were kept on ice and brought to the laboratory less than 1 hr after their removal. Chromaffin cells were isolated following a few modifications of the procedures of Hochman and Perlman [17] and Schneider *et al.* [18]. Briefly, the medullae of six to eight glands were dissected free of cortical tissue and

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† Abbreviations: ACh, acetylcholine; cyclic GMP, cyclic guanosine 3′,5′-monophosphate; CA, catecholamine; IMX, 3-isobutyl-1-methylxanthine; McN-A-343, 4-(*m*-chlorophenylcarbamoryloxy) - 2 - butyryltrimethylammonium chloride; AHR-602, *N*-benzyl-3-pyrrolidyl acetate methobromide; and ED_{50}, effective dose which produces half-maximal response.

placed in ice-cold calcium-free Krebs–Ringer buffer, pH 7.4 (118 mM NaCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 10 mM glucose, and 0.5% bovine serum albumin: buffer I). The medullary tissue was cut into small pieces (≈2 mm), washed with 30 ml of buffer I three times, and then submitted to four successive 30-min digestions with collagenase (1.5 mg/ml, CLS type I from Worthington) in the same buffer, at 37°. The dispersed cells were removed after each digestion and filtered through four layers of cheesecloth. The first harvest containing mostly red blood cells was discarded, and the three others were pooled and washed three times with 30 ml of buffer I supplemented with 2.2 mM $CaCl_2$ (buffer II). Chromaffin cells were then isolated by a 2-hr sedimentation in 40 ml of buffer II at room temperature and normal gravity. During this process, chromaffin cells sedimented at the bottom of the tube while lymphocytes and debris remained mostly in the supernatant fraction. The supernatant fraction was discarded, and chromaffin cells were resuspended in 6 ml of buffer II containing 100 units penicillin and 100 μg streptomycin. The cells were then gassed with a mixture of 5% CO_2 and 95% O_2 and stored overnight at room temperature. The next day, the cells were washed with 30 ml of buffer II and counted in a hemocytometer. Viability (over 95 per cent) was determined by the exclusion of trypan blue. Fifty to sixty million chromaffin cells were usually obtained by such a procedure.

Stimulation of catecholamine secretion. CA secretion was assayed as described previously [16] by the addition of 2.5×10^5 chromaffin cells (0.1 ml) to a prewarmed (37°) solution (0.9 ml) of buffer II containing the various drugs to be tested. The reaction was stopped after 5 min by transferring the tubes to an ice–water bath. The cells were centrifuged at 110 *g* for 10 min, and 0.7-ml aliquots of the supernatant fractions were collected. The samples were mixed with 70 μl of cold 50% trichloroacetic acid and were centrifuged at 900 *g* for 30 min. The resulting supernatant fractions were collected and stored at −20° for CA determination. Secretory experiments were performed in triplicate, and controls were obtained by incubation of the cells in the absence of drugs at 0° and 37°. The basal secretion was obtained by subtracting the release in the absence of drug at 0° from the release at 37°.

Measurement of catecholamines. For total CA determination (epinephrine plus norepinephrine), aliquots (200 μl) were neutralized with 1N NaOH (14 μl) and subjected to the oxidation procedure of Miura *et al.* [19]. Fluorescence was monitored on a spectrofluorometer (Carl Zeiss, model AFMa) at excitation/emission wavelengths of 410/515 nm respectively. At these wavelengths, the emission intensities of epinephrine and norepinephrine were equal at equal concentrations. Each experiment was repeated four times in triplicate, and values are expressed as nmoles of CA secreted per 10^6 cells per 5 min.

Cyclic nucleotide assay. Cyclic nucleotides were assayed as follows. Chromaffin cells (5×10^5, 0.1 ml) were added to a pre-warmed (37°) solution of buffer II (0.9 ml) containing the various drugs to be tested. The reaction was stopped after 3 min by the addition of 1 ml of cold 20% trichloroacetic acid. Cells were broken by freezing and thawing three times, and the white precipitate was removed by centrifugation at 900 *g* for 30 min (0–4°). The supernatant fractions were placed in a boiling-water bath for 3 min and extracted five times with 4 ml portions of water-saturated ether. After evaporation *in vacuo*, the samples were dissolved with 0.3 ml of water and stored at −20° for cyclic nucleotide determination. Assays were performed in duplicate, and controls were obtained by the incubation of the cells in the absence of drugs.

Cyclic nucleotide determination. Cyclic AMP and cyclic GMP were measured from 10 and 30 μl aliquots, respectively, by the radioimmunoassay techniques of Frandsen and Krishna [20]. Recovery (95 per cent) was calculated by the addition of 2000 cpm of [^{3}H]cyclic GMP to the samples before the ether extraction step described previously. When the ether extracts were passed through columns (0.5 × 5 cm) of Dowex AG1-X8 (formate form) [21] before the measurement of cyclic nucleotides, the recovery was 65 per cent. Cyclic GMP was routinely measured without prior chromatography on Dowex AG1-X8, and the non-specific readings (values obtained after digestion of the samples with phosphodiesterase, Sigma Chemical Co., St. Louis, MO) were smaller than 5 per cent of the amount of cyclic nucleotide contained in the samples. Values are expressed as pmoles of cyclic GMP per 10^6 cells.

Materials. [^{3}H]Cyclic GMP was purchased from the New England Nuclear Corp. (Boston, MA). Cyclic GMP [^{125}I]succinyl cyclic GMP tyrosine methyl ester, and anticyclic GMP antiserum were obtained from Schwarz-Mann (Orangeburg, NY) as assay kits. ACh, nicotine, hexamethonium, muscarine, isobutylmethylxanthine, atropine, dopamine, propanolol, adrenaline, noradrenaline, histamine, and serotonin were purchased from the Sigma Chemical Co. Oxotremorine was a product of the Aldrich Chemical Co. (Milwaukee, WI). Cyclic GMP, dibutyryl cyclic GMP, and 8-bromo cyclic GMP were obtained from Boehringer Mannheim. Furthretonium iodide (Professor E. Ariens, Nijmegen), McN-A-343 (McNeil Labs, Inc., Pittsburg, PA, U.S.A.), and AHR-602 (A. H. Robins, Co., Richmond, VA, U.S.A.) were gifts to our laboratory.

RESULTS

Concentration-dependent increase in cyclic GMP levels and catecholamine secretion in response to cholinergic agonists. Figures 1 and 2 illustrate the effects of ACh, nicotine, and muscarine on cyclic GMP levels and CA secretion in our preparation of bovine adrenal chromaffin cells. ACh induced a dose-dependent accumulation of cyclic GMP levels (intra- plus extracellular) with a half-maximum stimulation observed at 0.1 μM (Fig. 1). Nicotine, a potent stimulator of CA secretion (ED_{50}: 2 μM, Fig. 2), had no stimulatory effect on cyclic GMP levels at concentrations between 10^{-8} and 10^{-3} M (Fig. 1). Conversely, muscarine, a potent stimulator of cyclic GMP levels (ED_{50}: 0.3 μM, Fig. 1), did not evoke any secretion of CA (Fig. 2). The maximum accumulation of cyclic GMP in response to ACh was com-

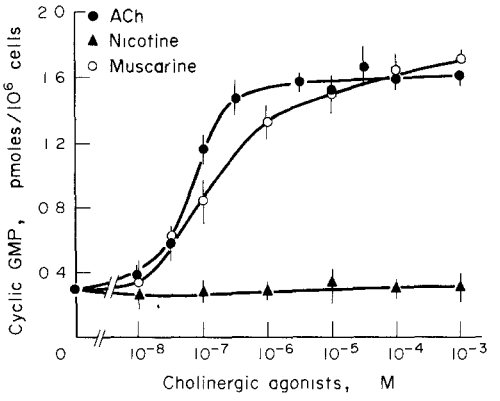

Fig. 1. Effects of increasing concentrations of ACh (●—●), nicotine (▲—▲), and muscarine (○—○) on cyclic GMP levels in isolated bovine adrenal chromaffin cells. Cells (0.5×10^6 in 1 ml of buffer II) were incubated for 3 min at 37° in the presence of the indicated concentrations of the cholinergic drugs, and GMP levels (intra- plus extracellular) were measured as described under Methods. The experiments were performed three times in duplicate, and values are means ± S.D.

parable to that obtained with muscarine (5-fold stimulation in Fig. 1) and was observed at a concentration of ACh (10^{-6} M) that had little effect on CA secretion (Fig. 2). Of interest was the observation that the ED_{50} of ACh for stimulation of cyclic GMP levels (0.1 μM) was 100 times smaller than that observed for stimulation of CA release (10 μM). These data clearly indicate the presence of two types of cholinergic receptors in our cell preparation, the nicotinic receptor being specifically involved in the stimulation of CA secretion and the muscarinic receptor in the regulation of cyclic GMP levels.

Relative potency of various muscarinic drugs in stimulating cyclic GMP levels. To define the type of muscarinic receptor involved in the cyclic GMP response and to compare it with the muscarinic receptor of other sympathetically innervated tissues [9], the relative potencies of various muscarinic agonists toward cyclic GMP stimulation were measured (Table 1). Acetylcholine was the most potent drug tested with an ED_{50} of 0.10 μM. It was closely followed by oxotremorine, methacholine, and muscarine (ED_{50}: 0.2, 0.25, and 0.3 μM respectively). Carbamylcholine, furthretonium, arecholine, and bethanechol were also potent stimulators of cyclic GMP levels. Their relative potencies in comparison with that of ACh, however, ranged between 2.9 and 0.3 per cent. On the other hand, pilocarpine, McN-A-343 and AHR-602, at concentrations between 10^{-8} and 10^{-3} M, had no stimulatory effect. Therefore, the stimulation of cyclic GMP levels in our preparation of bovine adrenal chromaffin cells was selectively and preferentially induced by some muscarinic drugs (ACh, oxotremorine, methacholine and muscarine), whereas other muscarinic agonists, such as pilocarpine, McN-A-343 and AHR-602, had no stimulatory effect.

Specificity of the cyclic GMP response to ACh. It was important to determine if the cyclic GMP response to ACh could be elicited by the stimulation of other drug receptors. Therefore, agonists and antagonists of various neurotransmitter receptors were tested in the absence or presence of ACh (Table 2). Incubation of the chromaffin cells in the presence of epinephrine, norepinephrine, dopamine, histamine, or serotonin, at 100 μM, did not affect the basal levels of cyclic GMP. Furthermore, phentolamine, propranolol, or haloperidol, at 10 μM, did not antagonize the stimulation of cyclic GMP levels in response to ACh (50 μM). These data indicate that the adrenergic, dopaminergic, histaminergic, and serotonergic receptors were not involved in the cholinergic stimulation of cyclic GMP levels.

Effects of phosphodiesterase inhibitors on cyclic nucleotide levels. Since cyclic nucleotides can be rapidly metabolized by intracellular phosphodiesterase, it was of interest to investigate the actions of

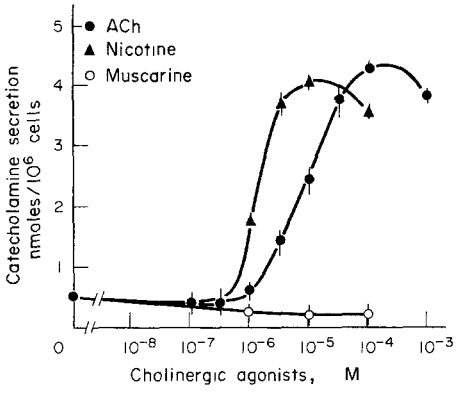

Fig. 2. Effects of increasing concentrations of ACh (●—●), nicotine (▲—▲), and muscarine (○—○) on CA secretion from isolated bovine adrenal chromaffin cells. Cells (0.25×10^6 in 1 ml of buffer II) were incubated for 5 min at 37° in the presence of the indicated concentrations of the cholinergic drugs, and the release of CA (adrenaline plus noradrenaline) was measured as indicated under Methods. The experiments were performed three times in triplicate, and values are means ± S.D.

Table 1. Relative stimulatory potencies of various cholinergic drugs on the levels (intra- plus extracellular) of cyclic GMP in isolated bovine adrenal chromaffin cells

Cholinergic drugs	ED_{50}* (μM)	Relative potency† (%)
ACh	0.1 ± 0.03	100
Oxotremorine	0.2 ± 0.02	50
Methacholine	0.25 ± 0.09	40
Muscarine	0.3 ± 0.04	33
Carbamylcholine	3.4 ± 1.1	2.9
Furthretonium	4.0 ± 0.9	2.5
Arecholine	4.5 ± 1.2	2.2
Bethanechol	38.3 ± 9.1	0.3
Pilocarpine	Inactive	
McN-A-343	Inactive	
AHR-602	Inactive	

* ED_{50}: concentration which gives half-maximum stimulation of cyclic GMP levels. Values (mean ± S.D.) were obtained from double reciprocal plots of dose–response curves performed three times in duplicate. Cyclic GMP was measured as described under Methods.

† Relative to ACh.

Table 2. Effects of receptor agonists and antagonists on basal and ACh-stimulated levels of cyclic GMP in isolated bovine adrenal chromaffin cells

Addition	Cyclic GMP	
	Pmoles/10^6 cells*	% Control
Control	0.36 ± 0.06	100
Epinephrine (100 μM)	0.46 ± 0.02	110
Norepinephrine (100 μM)	0.41 ± 0.03	114
Dopamine (100 μM)	0.39 ± 0.08	108
Histamine (100 μM)	0.32 ± 0.04	89
Serotonin (100 μM)	0.35 ± 0.07	97
ACh (50 μM)	1.15 ± 0.15	320
ACh + phentolamine (10 μM)	1.12 ± 0.08	310
ACh + propranolol (10 μM)	1.13 ± 0.09	314
ACh + haloperidol (10 μM)	1.16 ± 0.12	322

* Experiments were performed three times in duplicate, and values are means ± S.D. Cyclic GMP was measured as described under Methods.

various phosphodiesterase inhibitors on their levels. Table 3 shows the effects of isobutylmethylxanthine (IMX), theophylline (The) and papaverine (Pap) on basal and ACh-stimulated levels of cyclic GMP and cyclic AMP. IMX (0.5 mM) was found to be a very potent inhibitor of cyclic GMP phosphodiesterase since it caused a 7.2-fold increase in cyclic GMP levels (from 0.48 to 3.48 pmoles/10^6 cells) and a 3-fold potentiation of cyclic GMP accumulation in response to ACh (from 2.4 to 7.2 pmoles/10^6 cells). Theophylline (0.5 mM) and papaverine (0.5 mM) had no, or little, stimulatory effect on basal and ACh-stimulated levels of cyclic GMP. On the other hand, stimulation with ACh had no effect on cyclic AMP levels. IMX (0.5 mM) only caused a 1.8-fold increase in cyclic AMP levels in the presence or absence of ACh. Theophylline and papaverine were also poor inhibitors of cyclic AMP phosphodiesterase (1.1- and 1.3-fold increase in cyclic AMP levels, respectively, in the presence of The and Pap).

Effect of low concentrations of ACh on nicotine-induced catecholamine secretion. Following our observation that the accumulation of cyclic GMP and the release of CA in response to ACh occurred via the stimulation of two distinct receptors and at two distinct ACh concentrations (Figs. 1 and 2), it seemed important to investigate the possible modulation of CA release by cyclic GMP. Therefore, the nicotine-induced CA secretion was measured in the presence and absence of ACh and IMX (Table 4). Co-stimulation of the muscarinic receptor with ACh at 0.2 and 0.5 μM induced a respective 19 and 33 per cent reduction of nicotine (50 μM)-induced CA secretion. The inhibitory effect of ACh on CA secretion was antagonized by atropine at 0.1 μM. The release of CA in response to nicotine was also inhibited 44 per cent in the presence of IMX, a specific inhibitor of phosphodiesterase (Fig. 3). When the chromaffin cells were preincubated with a mixture of IMX and ACh, prior to stimulation with nicotine, the inhibitory effects of both drugs were additive. Atropine selectively antagonized the action of ACh, leaving intact the inhibitory effect of IMX. These data clearly demonstrate the involvement of a muscarinic receptor in the inhibitory action of ACh and suggest a mediator role for cyclic GMP in this effect.

Effect of exogenous dibutytyl cyclic GMP on catecholamine secretion. Basal and nicotine-induced CA secretions were also measured in the presence of

Table 3. Effects of various phosphodiesterase inhibitors on basal and acetylcholine-stimulated levels of cyclic GMP and cyclic AMP in isolated bovine adrenal chromaffin cells

Addition*	Cyclic GMP		Cyclic AMP	
	Pmoles/10^6 cells†	% Control	Pmoles/10^6 cells†	% Control
Control	0.48 ± 0.08	100	7.7 ± 0.7	100
ACh (50 μM)	2.40 ± 0.24	500	7.7 ± 0.7	100
ACh + IMX (0.5 mM)	7.20 ± 0.60	1500	14.1 ± 1.2	183
ACh + The (0.5 mM)	3.02 ± 0.28	630	8.6 ± 1.6	111
ACh + Pap (0.5 mM)	2.16 ± 0.20	450	8.4 ± 1.7	109
IMX (0.5 mM)	3.48 ± 0.36	725	13.8 ± 1.5	180
The (0.5 mM)	1.20 ± 0.08	250	8.6 ± 1.5	111
Pap (0.5 mM)	1.05 ± 0.09	220	10.3 ± 0.5	134

* Abbreviations: ACh, acetylcholine; IMX, 3-isobutyl-1-methylxanthine; The, theophylline; and Pap, papaverine.

† Experiments were performed three times in duplicate, and values are means ± S.D. Cyclic nucleotides were measured as described under Methods.

Table 4. Effects of low concentrations of ACh, IMX, and a mixture of ACh plus IMX on nicotine-induced catecholamine secretion from isolated bovine adrenal chromaffin cells

Addition*	Catecholamine secretion† (nmoles/10^6 cells)	Inhibition (%)
Nicotine (10 μM)	4.33 ± 0.03	0
Nicotine + ACh (0.2 μM)	3.50 ± 0.05	19
Nicotine + ACh (0.5 μM)	2.89 ± 0.07	33
Nicotine + ACh (0.2 μM) + Atr (0.1 μM)	4.35 ± 0.02	0
Nicotine + ACh (0.5 μM) + Atr (0.1 μM)	4.17 ± 0.06	4
Nicotine + IMX (1 mM)	2.42 ± 0.02	44
Nicotine + IMX (1 mM) + ACh (0.2 μM)	2.08 ± 0.08	52
Nicotine + IMX (1 mM) + ACh (0.5 μM)	1.17 ± 0.02	73
Nicotine + IMX (1 mM) + ACh (0.2 μM) + Atr (0.1 μM)	2.37 ± 0.05	45
Nicotine + IMX (1 mM) + ACh (0.5 μM) + Atr (0.1 μM)	2.42 ± 0.03	44

* Abbreviations are: ACh, acetylcholine; Atr, atropine; and IMX, isobutylxanthine.

† Experiments were performed as described under Methods with the exception that there was a 5-min preincubation (37°) of the cells in the presence of ACh, Atr or IMX before the stimulation with nicotine. "Catecholamine secretion" represents the amount of catecholamines released by nicotine after subtracting the base line release of catecholamines (0.5 nmole/10^6 cells per 5 min). Assays were repeated three times in triplicate, and values are means ± S.D.

increasing concentrations of dibutyryl cyclic GMP (Fig. 3). Exogenous dibutyryl cyclic GMP did not affect the basal levels of CA secretion but inhibited, in a dose-dependent manner (between 10^{-5} and 10^{-3} M), the secretion of CA induced by nicotine (50 μM). The half-maximum inhibition was observed at approximately 0.5 mM dibutyryl cyclic GMP. Similar inhibition of nicotine-induced catecholamine secretions was also observed in the presence of 8-bromo cyclic GMP (data not shown).

DISCUSSION

Previous studies have indicated that the cholinergic stimulations of cyclic GMP levels and CA secretion in isolated bovine adrenal chromaffin cells are rapid and ACh-dose dependent [16]. Taken alone, these observations suggest a mediator role for cyclic GMP in the stimulus–secretion coupling at this level. The present data (Figs. 1 and 2) and those obtained in *in vivo* [22] and *in vitro* [23] experiments, however, clearly indicate that the increase in cyclic GMP levels and the stimulation of CA secretion in response to cholinergic drugs occur through the stimulation of muscarinic and nicotinic receptors respectively. Therefore, the secretory effect of ACh cannot be mediated by cyclic GMP. There remains the possibility that the muscarinic stimulation of cyclic GMP levels modulates the nicotinic stimulation of CA secretion.

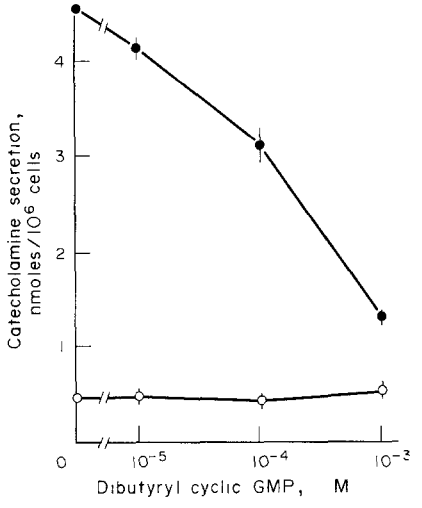

Fig. 3. Effects of increasing concentrations of dibutyryl cyclic GMP on basal and nicotine-stimulated release of CA from isolated bovine adrenal chromaffin cells. Cells (0.25×10^6/ml in buffer II) were incubated at 37° for 5 min with increasing concentrations of dibutyryl cyclic GMP, as indicated, in the presence (●——●) or absence (○——○) of nicotine (50 μM), and the release of CA (adrenaline plus noradrenaline) was measured as indicated under Methods. The experiments were repeated three times in triplicate, and values are means ± S.D.

The involvement of a muscarinic receptor in the cyclic GMP response to ACh is consistent with the muscarinic stimulation of cyclic GMP in other tissues [24]. The recent findings of a high affinity muscarinic binding site in homogenates of bovine adrenal medulla [13] and of guanylate cyclase in the plasma membrane of bovine adrenal chromaffin cells [25] strengthen the concept of a possible role for the muscarinic receptor in our cell preparation. Furthermore, the involvement of receptors other than the muscarinic one in the cyclic GMP response to ACh can almost be excluded since dopaminergic, adrenergic, serotonergic, and histaminergic drugs had no effect on basal and ACh-stimulated cyclic GMP levels (Table 2).

The inhibitory effect of dibutyryl cyclic GMP on CA secretion (Fig. 3) is in accord with the findings obtained in the rat pineal gland which indicate that lipophilic analogs of cyclic GMP inhibit the release of [^{3}H]noradrenaline [26, 27]. Furthermore, IMX and 8-bromo cyclic GMP have also been shown to inhibit the contraction of the guinea pig vas deferens evoked by transmural nerve stimulation [28]. Therefore, the inhibition of nicotine-induced CA release by experimental conditions (dibutyryl cyclic GMP: Fig. 3; IMX or low concentrations of ACh: Table 4) that elevate cyclic GMP without much affecting cyclic AMP in our cell preparation may be a feature common to other sympathetically innervated tissues.

The muscarinic inhibition of nicotine-induced CA secretion (Table 4) is in agreement with the finding of a muscarinic mechanism for inhibition of noradrenaline output from nerve terminals in a variety of tissues including cat spleen, guinea pig vas deferens, rabbit heart, and rabbit ear artery [29–33]. Muscarinic drugs were shown to inhibit the release of noradrenaline in response to nicotinic agents [34], and it was postulated that pre-synaptic inhibitory receptors are present in adrenergic nerve terminals. Such inhibitions have been reported to occur at concentrations of ACh that had no effect on the post-synaptic nicotinic receptors [33]. Of interest is the observation that the half-maximum stimulation of cyclic GMP levels in our cell preparation occurs at a concentration of ACh 100-fold smaller (10^{-7} M) than that required for half-maximum stimulation of CA release (10^{-5} M; Figs. 1 and 2). Furthermore, the orders of potency of various muscarinic agonists in stimulating cyclic GMP levels in our cell preparation (Table 1) and inhibiting [^{3}H]noradrenaline output from rabbit heart preparations [29] are quite similar. In both systems, ACh, oxotremorine, methacholine and muscarine were very potent, whereas pilocarpine, AHR-602 and McN-A-343 had no, or little, effect.

In conclusion, our results support the concept of a muscarinic regulation of CA secretion in bovine adrenal medulla and indicate a mediator role for cyclic GMP. Further studies are still needed to establish the mechanism of action of cyclic GMP and the possible physiological role of the muscarinic receptor. The use of isolated chromaffin cells in this regard seems to be promising.

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