MUSCARINIC RECEPTOR-MEDIATED INCREASE IN CYCLIC GMP LEVEL IN ISOLATED BOVINE ADRENAL MEDULLARY CELLS

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1. Introduction

Adrenal medulla is regarded as a model of neurons containing catecholamine (CA), and is the most suitable tissue for studying the regulation of CA release and synthesis. Recently, some investigations have been carried out on these problems using isolated adrenal medullary cells [1–4]. Such preparations are suitable for use in elucidating how stimulation of membrane receptors is coupled with release and synthesis of CA. We have also isolated bovine adrenal medullary cells, which respond to acetylcholine (ACh), nicotine or excess K^+ (56 mM), by release of CA and increased synthesis of CA [5].

Here, we examine the effect of cholinergic agents and of excess K^+ on the level of cyclic GMP (cGMP) in the isolated cells and found that the cGMP level was increased via stimulation of muscarinic ACh receptors. The increase in cGMP level is considered in relation to the release of CA from the cells.

During preparation of this manuscript Schneider et al. [6] reported that the level of cGMP could be increased by ACh at lower concentrations than that required to initiate the release of CA from the isolated cells.

2. Methods

Bovine adrenal medullary cells were isolated in large quantity by sequential digestion of adrenal medullary slices with collagenase as in [5]. The isolated cells (~2×10^{-6} cells/ml) were incubated at 37°C with or without test compounds in Krebs-Ringer phosphate (KRP) buffer (pH 7.4) containing 0.5% bovine serum albumin, in 2 ml final vol. When 56 mM K^+ was used, the Na^+ content was decreased to keep the tonicity of the KRP buffer constant.

For determination of cGMP, the reaction was stopped by addition of 0.2 ml ice-cold 70% trichloroacetic acid. cGMP was separated on an ion exchange column (AG 50 W × 8, H-type, 200–400 mesh) [7] and measured by radioimmunoassay (assay kit). For determination of CA release, after incubation, the tubes were rapidly chilled in an ice-water bath, then centrifuged at 600 × *g* for 5 min in the cold. CA in the cells and medium was extracted with 0.4 N perchloric acid and measured fluorimetrically [8].

3. Results and discussion

ACh (3×10^{-6}) was found to increase the level of cGMP ~5-fold in isolated bovine adrenal medullary cells. As shown in fig.1, increase in the cGMP level was observed after incubation with ACh for only 30 s, the shortest time examined. The maximum cGMP level was observed after incubation for 2 min, and then the level decreased rapidly to normal. In the presence of 3-isobutyl-1-methyl-xanthine (IBMX, 0.3 mM) a phosphodiesterase inhibitor, the maximum level of cGMP was observed after 2–5 min incubation, then decreased more slowly.

The increase in cGMP level caused by ACh was dependent on the presence of extracellular Ca^{2+}; it was abolished by omission of Ca^{2+} from the medium and increased linearly with increase in concentration of Ca^{2+} to ~2 mM (data not shown).

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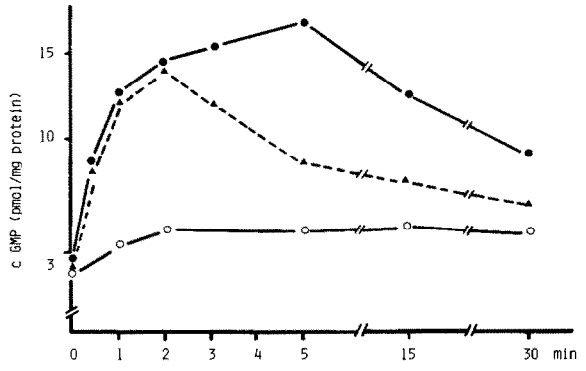

Fig.1. Time course of the effect of ACh on the level of cGMP in isolated bovine adrenal medullary cells. Cells were incubated in the presence or absence of 3×10^{-6} M ACh for the indicated periods. When the phosphodiesterase inhibitor IBMX (0.3 mM) was added, cells were preincubated with IBMX for 10 min then ACh was added, and incubations were continued for the indicated times. (○———○) Control; (▲– – –▲) IBMX (–); (●———●) IBMX (+).

Figure 2 shows the dose–response curve for the effect of ACh on the cGMP level. Increase in the cGMP level was detectable with as low as 10^{-8} M ACh, and the maximal response was observed with 10^{-6} M ACh. The half-maximal response was obtained at $\sim 2 \times 10^{-7}$ M ACh. It is of interest to compare this dose–response curve with that for the effect of ACh on the release of CA from the cells. Release of CA occurred at $>10^{-6}$ M ACh, the concentration for the maximal increase in the cGMP level, and was maximal at 10^{-4} M ACh. These findings are quite in agreement with [6] and are interesting in relation to the possible role of cGMP in regulation of CA release.

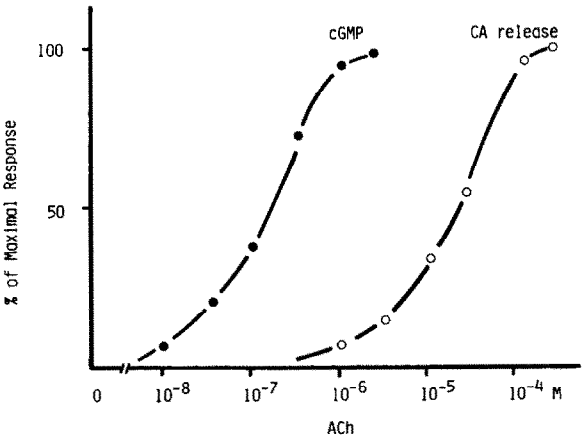

Fig.2. Dose–response curves for ACh-induced increase in cGMP level and ACh-induced CA release. Cells were preincubated for 10 min with IBMX (0.3 mM) then ACh was added, and incubations were continued for 5 min (for determination of cGMP) or 1 min (for determination of CA release). Maximal response: cGMP = 15 pmol/mg protein; CA release = 11–12% of the total intracellular CA content.

Figure 3 shows the effects of cholinergic agents and of excess K^+ on the cGMP level and release of CA from the cells. Increase in the cGMP level caused by ACh was inhibited by atropine, a muscarinic antagonist, but not by hexamethonium, a nicotinic antagonist, indicating that the ACh-induced increase in the cGMP level involved an interaction of ACh with muscarinic ACh receptors rather than nicotinic ACh receptors. On the other hand, the release of CA from the cells caused by ACh was inhibited by hexamethonium. Atropine also inhibited the release of CA, suggesting that atropine interacts with nicotinic ACh receptors, since it is well known that the release of CA from bovine adrenal medulla

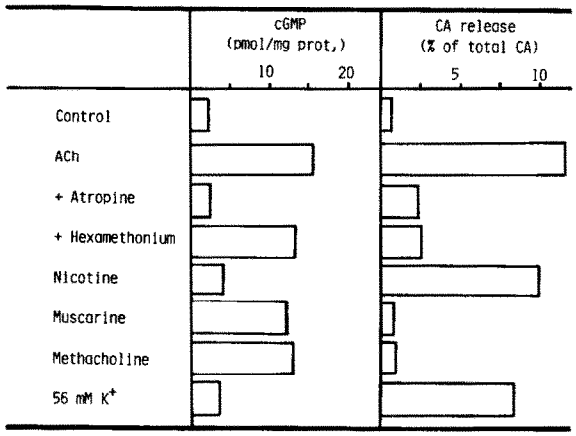

Fig.3. Effects of cholinergic agents and excess K^+ on the level of cGMP and release of CA. Cells were preincubated for 10 min with or without cholinergic antagonist (3×10^{-4} M) in the presence of IBMX (0.3 mM), then ACh (10^{-4} M) or an other cholinergic agonist (10^{-4} M) was added, and incubations were continued for 5 min (for cGMP) or 1 min (for CA release). To test the effect of excess K^+, the second incubation was carried out in medium containing 56 mM K^+.

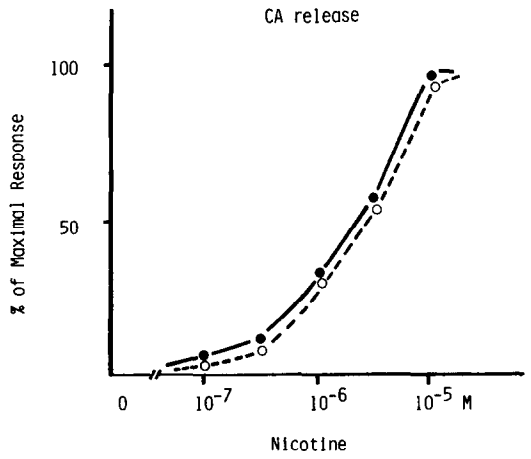

Fig.4. Dose–response curve for nicotine-induced CA release and effect of muscarine on it. Cells were preincubated for 10 min with or without muscarine (10^{-5} M) in the presence of IBMX (0.3 mM), then various concentrations of nicotine were added and the incubations were continued for 1 min. Maximal CA release represented 10% of the total intracellular CA content. (•———•) Nicotine; (o– – –o) nicotine + muscarine.

is mediated predominantly by nicotinic ACh receptors [9].

The mediation of muscarinic ACh receptors in the ACh-induced increase in the cGMP level was also indicated from results with other cholinergic agonists. The muscarinic agonists muscarine and methacholine caused 4–5-fold increase in the cGMP level, but they did not cause release of CA. On the contrary, nicotine or excess K^+, which caused release of CA, did not significantly alter the level of cGMP.

It does not appear from these results that there is a parallel relation between increase in the cGMP level in the cells and release of CA from the cells.

Next, we examined the possibility that the increase in cGMP via stimulation of muscarinic ACh receptors may inhibit the release of CA mediated by stimulation of nicotinic ACh receptors. Figure 4 shows the dose–response curve for nicotine-induced CA release from the isolated cells. The effective concentration range of nicotine for release of CA was found to be lower than that of ACh. However, the release of CA caused by various concentrations of nicotine was not affected by the presence of muscarine, which increased the level of cGMP in the cells.

The physiological role of cGMP in bovine adrenal medullary cells is unknown. The role of cGMP in regulation of CA synthesis is now under investigation.

Acknowledgements

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