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The release of ^{3}H-1-methyl-4-phenylpyridinium from bovine adrenal chromaffin cells is modulated by somatostatin

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Abstract

Besides cholinergic regulation, catecholamine secretion from adrenal chromaffin cells can be elicited and/or modulated by noncholinergic neurotransmitters and hormones. This study was undertaken to investigate the influence of somatostatin and octreotide on [^{3}H]MPP^{+} secretion evoked by KCl or cholinergic agents, from bovine adrenal chromaffin cells.

The release of [^{3}H]MPP^{+} was markedly increased by excess KCl (50 mM), acetylcholine (50 μM–10 mM) and by the nicotinic agonists, nicotine (5–100 μM) and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, 10–100 μM), but not by the muscarinic agonist, pilocarpine (10–100 μM). Acetylcholine-evoked release of [^{3}H]MPP^{+} from these cells was mainly mediated by nicotinic receptors: a) nicotine and DMPP stimulated the release of [^{3}H]MPP^{+}, b) a nicotinic antagonist, hexamethonium, markedly blocked the acetylcholine-evoked response and c) pilocarpine was devoid of effect on [^{3}H]MPP^{+} secretion. At all concentrations tested, somatostatin and octreotide interfered neither with [^{3}H]MPP^{+} basal release nor with KCl-induced release of [^{3}H]MPP^{+}. However, somatostatin (0.01–0.3 μM) increased the release of [^{3}H]MPP^{+} induced by a high concentration of acetylcholine (10 mM). Octreotide (1–10 μM) had no effect.

These results, showing that somatostatin potentiates acetylcholine-induced [^{3}H]MPP^{+} release, support the hypothesis that somatostatin may increase the release of catecholamines from adrenal medullary cells.

Keywords: Bovine adrenal chromaffin cells; Somatostatin; Octreotide; MPP^{+} release

1. Introduction

Catecholamines (CA) released by the sympathoadrenal system (the sympathetic nervous system and the adrenal medulla) are involved in the regulation of many physiological functions, particularly in the "fight or flight" syndrome, a stress-response in dealing with emergencies. Adrenaline (AD) (and, in a lesser extent, noradrenaline (NA)), released from the adrenal medulla into the bloodstream, is a critical mediator of that response, acting as a hormone, and affecting many organs and tissues throughout the body.

The adrenal chromaffin cells synthesise and secrete, into the bloodstream, CA, mostly AD and NA. They have the ability to accumulate circulating amines, as well as similar compounds, against a concentration gradient [1].

Chromaffin cells can be isolated from the bovine adrenal medulla with relative homogeneity, and purified in extremely large quantities. Chromaffin cells primary cultures have become an important research tool to study basic processes that regulate CA synthesis, storage and release [1].

1-Methyl-4-phenylpyridinium (MPP^{+}), the major metabolite of the exogenous neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), produces a parkinsonian syndrome in humans, monkeys and various other animals by selectively destroying the nigrostrial dopaminergic neurons. This neurotoxin is often used as a model substrate for CA studies [2–10], since it shares with these amines, not only structural and functional properties, but also the monoaminergic transporters: the neuronal NA (NAT) and dopamine (DAT) transporters [11–13], the extraneuronal monoamine transporter, EMT, also known as OCT3 [2,4,14], the organic cation transporter type 1, OCT1 [14], the organic cation transporter type 2, OCT2 [14], and the vesicular catecholamine transporter of chromaffin granules, VMAT [15]. In

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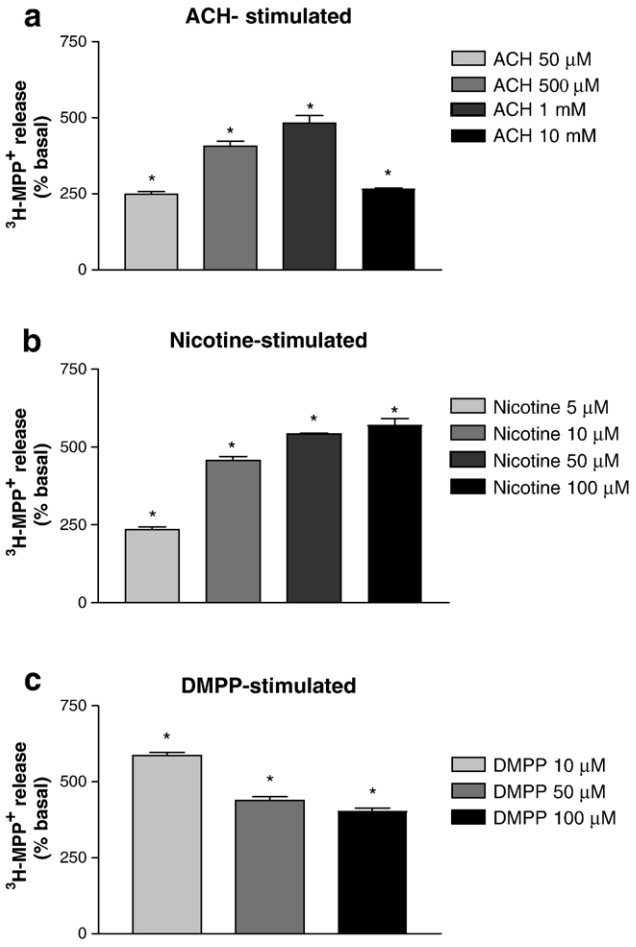

Fig. 1. Effect of acetylcholine (ACH; 50 µM–10 mM; n=3–31) (a), nicotine (5–100 µM; n=4–12) (b) and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP; 10–100 µM; n=4) (c) upon [^{3}H]MPP$^+$ release from bovine chromaffin cells. For these studies, cells were previously labelled with [^{3}H]MPP$^+$ (200 nM) for 60 min, washed out, preincubated for 10 min, and then incubated for 15 min with or without (control) the drugs. Shown are arithmetic means±SEM. *P<0.05, compared to the corresponding control.

addition, MPP$^+$ has the advantage of not being subjected to metabolism [16] or to spontaneous oxidation, so that the use of enzyme inhibitors and ascorbic acid is avoided.

Somatostatin (SS), a peptide hormone isolated by Brazeau et al. [17], and initially described as an inhibitor of growth hormone release from the pituitary gland, is known by its inhibitory effects on a variety of biological functions [18]. The presence of SS [19] and its receptors [20] in human adrenal medulla suggests that this peptide may play a role in the physiological control of CA secretion, acting as a local factor, via autocrine or paracrine mechanisms.

Among the several synthetic SS analogues developed, octreotide has been the most extensively studied, because of several advantageous characteristics, when compared to the native peptide: greater potency, longer duration of action and remarkable metabolic stability [21].

In the present work, we investigated the effects of SS or octreotide on [^{3}H]MPP$^+$ release, induced by high KCl or cholinergic agents, from bovine adrenal chromaffin cells.

2. Materials and methods

2.1. Preparation of adrenal medullary cells

Bovine adrenal glands were obtained from a local slaughterhouse and were processed for cell culture within 2 h of slaughter, being transported to the laboratory in ice-cold Ca^{2+}–Mg^{2+}-free buffer A (containing 154 mM NaCl, 2.6 mM KCl, 0.85 mM KH_2PO_4, 2.15 mM K_2HPO_4, 10 mM glucose and 12.7 mM HEPES, pH 7.4). The chromaffin cells were isolated by digestion with collagenase A as described by others [1,22] with minor modifications. Briefly, the adrenal glands were trimmed of fat and washed with Ca^{2+}–Mg^{2+}-free buffer A. The buffer was injected into the adrenal vein to rinse out the remaining blood from the gland, which was then digested with a Ca^{2+}–Mg^{2+}-free buffer A solution containing 0.25% collagenase A and 0.01% DNAse I. Five ml of this solution was injected into the adrenal vein of each gland. This procedure was repeated every 15 min with the glands incubated at 37 °C in buffer A. After 45 min, the digested medullary tissue was separated mechanically from the adrenal cortex and incubated in buffer A, containing collagenase, for 30 min at 37 °C. Subsequently, they were filtered through a 200-µm nylon mesh. The filtered cells were washed 3× with Ca^{2+}–Mg^{2+}-free buffer to remove the collagenase/DNAse and then filtered through a 100-µm nylon mesh.

2.2. Cell culture

Chromaffin cells were cultured in Dulbecco's Modified Eagle's medium/F12 Ham supplemented with 10% foetal calf serum, 2 mM L-glutamine, 15 mM HEPES, 10 µM cytosine arabinoside, 100 units/ml penicillin, 100 µg/ml streptomycin and 0.25 µg/ml amphotericin B (Sigma, St. Louis, MO, USA). The cells were plated in collagen-coated 24-well plastic culture dishes (2 cm^2; ∅ 15.6 mM; Corning Costar, Corning, NY, USA) at a density of 4–5×10^5–10^6 cells/well and incubated at 37 °C in an atmosphere consisting of 5% CO_2 in air. Culture medium was replaced every 24 h and, in the day before the experiment, was made free of foetal calf serum. For the experiments, viable cells (>90% by trypan blue) were used after 4 days in culture.

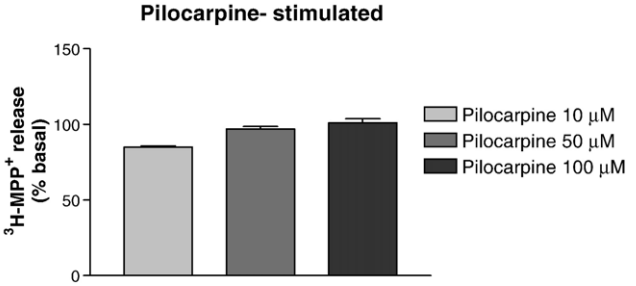

Fig. 2. Effect of pilocarpine (10–100 µM; n=6–8) upon [^{3}H]MPP$^+$ release from bovine chromaffin cells. For these studies, cells were previously labelled with [^{3}H]MPP$^+$ (200 nM) for 60 min, washed out, preincubated for 10 min, and then incubated for 15 min with or without (control) the drug. Shown are arithmetic means±SEM.