Effect of somatostatin on the release of adrenaline and noradrenaline from bovine adrenal chromaffin cells.

Laura Ribeiro[a,b], Fátima Martel[a] and Isabel Azevedo[a]

a. Department of Biochemistry.
b. Institute of Pharmacology and Therapeutics. Faculty of Medicine. University of Porto. Portugal.

Correspondence: Dr: Laura Ribeiro. Department of Biochemistry, Faculty of Medicine, 4200-319 Porto, Portugal.
Phone: 351-22-5095694; FAX: 351-22-5502402; Email: lribeiro@med.up.pt

Cell Biology of the Chromaffin Cell
R. Borges & L. Gandía Eds.
Instituto Teófilo Hernando, Spain, 2004

Bovine chromaffin cells are innervated by the splanchnic nerve, which, upon stimulation, releases acetylcholine (ACh), which, in turn, triggers catecholamine (CA) secretion. It has become clear that, besides the cholinergic regulation, adrenal chromaffin cells are under the control of a large number of noncholinergic factors that can evoke or modulate CA secretion.

There is strong evidence that adrenaline (AD) and noradrenaline (NA) are stored in two different types of cells[1] and that these two cell subpopulations may be selectively activated[2, 3].

Somatostatin (SS) acts as a neurotransmitter and neuro-modulador in the central nervous system and in the periphery, producing multiple effects, through interactions with five specific membrane receptors[4]. SS effects on the adrenal medulla secretion are largely unknown.

The aim of our work was to test the hypothesis of SS exerting a modulatory effect over cholinergic-evoked CA release from bovine adrenal chromaffin cells.

The chromaffin cells were isolated by digestion with collagenase A, cultured in DMEM/F12 Ham supplemented with 10% foetal calf serum, and plated in collagen-coated 24-well plastic culture dishes at a density of 4-5 x 10^5-10^6 cells/well. For the experiments, cells were used after 4 days in culture. For studies on CA release, cells were preincubated for 10 min, to measure basal secretion, and incubated for 15 min under basal conditions or under different experimental conditions. CA in the cells and liquids were quantified by means of high pressure liquid chromatography with electrochemical detection (HPLC-ED). The cellular content ratios of AD/NA differed among different cultures, which were grouped in: AD-rich cell cultures (when AD/NA = 2.4-2.5) and AD-poorer cell cultures (when AD/NA = 1.2-1.7).

RESULTS AND DISCUSSION

Adrenal chromaffin cells synthesised and accumulated large amounts of AD and NA. Acetylcholine (ACh), 50 μM-10 mM, increased, in a concentration-dependent manner, the release of CA from both cell cultures. At concentrations of 500 μM and 1 mM, ACh released CA preferentially from AD-poorer cultures.

Similarly, nicotine (5-100 μM) and dimethyl-phenyl-piperazinium (DMPP) (10-100 μM), two selective nicotinic agonists, caused a predominant CA release from AD-poorer cultures.

The release of both CA, elicited by ACh, was significantly reduced by hexamethonium (100 μM), a selective nicotinic antagonist, only from the AD-poorer cultures.

The muscarinic receptor antagonist, atropine (100 μM), had no significant effect on the ACh-evoked CA release from AD-poorer cultures, whereas it inhibited the release of both CA from AD-rich cultures.

Somatostatin was found to increase the ACh-evoked CA release from the AD-rich cultures but was unable to significantly affect the release of CA from AD-poorer cultures. On the other hand, it attenuated the CA secretion from the AD-poorer cultures induced either by nicotine or DMPP.

Taken together these results suggest that: 1) AD-rich and AD-poorer cell cultures respond differentially to muscarinic and nicotinic stimulation; 2) Somatostatin increases the AD/NA ratio in chromaffin cell secretion through a differential effect on AD-rich cultures (increase), probably by interaction with muscarinic receptors, and on AD-poorer cultures (decrease), probably by interaction with nicotinic receptors.

It is generally accepted that cholinergic agents evoke endogenous CA release, from adrenal chromaffin cells, through nicotinic receptors. However, there are some reports, either from isolated perfused adrenal gland[5], or from primary chromaffin cell cultures[6], showing that muscarinic receptors may also be involved in CA secretion. Our results strongly indicate that a muscarinic response comes mainly from AD-rich cells, whereas the other cell type is mainly responsible for the cholinergic evoked CA release. This may explain some difficulty in visualising the muscarinic component in acetylcholine evoked CA release from a mixture of adrenal chromaffin cells.

Although SS is a well-known inhibitory peptide, there are a few reports of this peptide acting as a positive modulator[7,8]. Interestingly, recent works[9,10] have described, in other experimental cell models, a cross talk between SS and other peptides, which also

signal via G_i/G_o-coupled receptors, and agents signalling via G_q-coupled receptors (like muscarinic agonists) leading to the potentiation of the release of several hormones. In addition, similarly to our findings, SS by itself did not affect secretion. The experiments described here have provided, to our knowledge, the first evidence for a positive role of somatostatin on adrenal chromaffin cells. This positive effect involves a specific increase in AD secretion.

ACKNOWLEDGEMENTS

Supported by FCT, POCTI (37278/FCB/2001) and FEDER.

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