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Differential activation of enkephalin, galanin, somatostatin, NPY, and VIP neuropeptide production by stimulators of protein kinases A and C in neuroendocrine chromaffin cells

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Abstract

Neuropeptides function as peptide neurotransmitters and hormones to mediate cell–cell communication. The goal of this study was to understand how different neuropeptides may be similarly or differentially regulated by protein kinase A (PKA) and protein kinase C (PKC) intracellular signaling mechanisms. Therefore, this study compared the differential effects of treating neuroendocrine chromaffin cells with stimulators of PKA and PKC on the production of the neuropeptides (Met)enkephalin, galanin, somatostatin, NPY, and VIP. Significantly, selective increases in production of these neuropeptides were observed by forskolin or phorbol myristate acetate (PMA) which stimulate PKA and PKC mechanisms, respectively. (Met)enkephalin production was stimulated by up to 2-fold by forskolin treatment, but not by PMA. In contrast, PMA treatment (but not forskolin) resulted in a 2-fold increase in production of galanin and somatostatin, and a 3-fold increase in NPY production. Notably, VIP production was highly stimulated by forskolin and PMA, with increases of 3-fold and 10–15-fold, respectively. Differences in elevated neuropeptides occurred in cell extracts compared to secretion media, which consisted of (i) increased NPY primarily in secretion media, (ii) increased (Met)enkephalin and somatostatin in secretion media (not cell extracts), and (iii) increased galanin and VIP in both cell extracts and secretion media. Involvement of PKA or PKC for forskolin or PMA regulation of neuropeptide biosynthesis, respectively, was confirmed with direct inhibitors of PKA and PKC. The selective activation of neuropeptide production by forskolin and PMA demonstrates that PKA and PKC pathways are involved in the differential regulation of neuropeptide production.

Keywords: Neuropeptide biosynthesis; Enkephalin; Galanin; Somatostatin; NPY; VIP; Forskolin; PMA; Protein kinase; Regulation; Chromaffin cells

1. Introduction

Peptide neurotransmitters and hormones, collectively known as neuropeptides, function in the nervous and endocrine systems to mediate cell–cell communication that is required for biological control. Neuropeptides mediate activity-dependent neurotransmission of information among neuronal circuits, and function as endocrine regulators of target cellular and organ systems (Krieger et al., 1983). Neuropeptides possess diverse physiological actions defined by their unique primary sequences, which includes the enkephalin opioid peptide that regulates analgesia (Gustein and Akil, 2006; Law

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et al., 2000) and immune functions (Hedner and Cassuot, 1987; Owens and Smith, 1987), galanin which regulates cognition (Steiner et al., 2001; Robinson, 2004), neuropeptide Y (NPY) which participates in regulating feeding behavior and blood pressure (Ramos et al., 2005; Waeber et al., 1990), somatostatin which mediates growth regulation (Norris, 1997), and vasoactive intestinal peptide (VIP) that regulates immune functions (Gomariz et al., 2001). Knowledge of the regulatory mechanisms that control the biosynthesis of neuropeptides is important for understanding biological control mechanisms that influence the physiological functions of neuropeptides.

Neuropeptides are produced and stored within secretory vesicles that undergo regulated secretion upon stimulation of neuroendocrine cells by receptor-mediated mechanisms. Intracellular second messenger signaling mechanisms mediate the regulation of neuropeptide production to replace intracellular stores of these biologically active peptides as they are secreted. Notably, the protein kinase A (PKA) and protein kinase C (PKC) pathways are known to regulate neuropeptide secretion (Sirianni et al., 1999; Giraud et al., 1991; Rokaeus et al., 1990; Magni and Barnea, 1992; Vlotides et al., 2004). Because neuropeptide secretion is linked to cellular production of neuropeptides, it is likely that PKA and PKC pathways may regulate neuropeptide production. Furthermore, comparison of PKA and PKC mechanisms in regulating neuropeptides is important for assessing whether similar or distinct PKA or PKC pathways regulate neuropeptide biosynthesis.

Therefore, the goal of this study was to evaluate the differential regulation of several neuropeptides during activation of PKA and PKC in neuroendocrine chromaffin cells in primary culture (prepared from bovine adrenal medulla). Cellular levels of the neuropeptides (Met)enkephalin, galanin, somatostatin, NPY, and VIP were assessed after treating chromaffin cells with forskolin or phorbol myristate acetate (PMA) that result in cellular activation of PKA and PKC, respectively. Activation of PKA results from forskolin stimulation of adenylate cyclase (Seamon et al., 1983) that catalyzes the production of cAMP, which stimulates cAMP-dependent PKA (Meinkoth et al., 1993; Lodish et al., 2000). Cellular PKC is activated by phorbol myristate acetate (PMA) that is known to directly stimulate PKC (Goel et al., 2007).

Results from this study illustrated three distinct, selective modes for PKA and PKC regulation of neuropeptide production. Total (Met)enkephalin production was stimulated primarily by forskolin, rather than by PMA. However, production of total cellular galanin, somatostatin, and NPY was preferentially stimulated by PMA, compared to forskolin. VIP production was extensively stimulated by both forskolin and PMA. Differences were observed in the location of increased neuropeptide with respect to changes in neuropeptide levels in cell extracts or secretion media. These novel findings demonstrate the selective regulation of neuropeptide production by PKA compared to PKC pathways in neuroendocrine chromaffin cells.

2. Experimental procedures

2.1. Treatment of chromaffin cells in primary culture with forskolin or PMA, combined with inhibitors of PKA or PKC

Primary cultures of bovine chromaffin cells were prepared from fresh adrenal medulla as described previously (O'Connor et al., 2007), plated at 4×10^5 cells/well to 2×10^6 cells/well (6-well plate). Experiments were conducted after 3–5 days in culture. Cell cultures at this time showed consistent numbers of cells/well, assessed by protein determination.

Experiments were conducted by treating cells with forskolin (50 μM) or phorbol myristate acetate (PMA, 100 nM) for 48 and 72 h in complete culture media (O'Connor et al., 2007); controls included incubation with vehicle control (0.001% DMSO). In addition, forskolin-treated cells were incubated with or without the protein kinase A inhibitor, KT-5720 (10 μM) (Hidaka and Kobayashi, 1992; Haddad et al., 2005), and PMA-treated cells were incubated with or without the protein kinase C inhibitor, bisindolylmaleimide I (Bis I) (Hidaka and Kobayashi, 1992), for 72 h.

At the end of each treatment period, cells were harvested and prepared for neuropeptide analyses, achieved by lysing cells in 0.1 N acetic acid that was prepared as an acid extract, as we have described previously (Miller et al., 2003). In addition, culture media was collected for measurement of neuropeptides secreted during the treatment period. The cell culture media included bacitracin (10 μM) as protease inhibitor. Total neuropeptide production during the treatment period was measured as the combined sum of neuropeptides in cell extracts and culture media.

Each experiment utilized replicate cell samples (triplicate), and experiments were conducted at last three times. Results are expressed as the amount of total neuropeptide measured from cell extracts and media per well, and expressed as the mean $x \pm$ s.e.m., with evaluation of statistical significance (Student's t-test, with $p < 0.05$ for significance).

It was noted that there was some variability among primary chromaffin cell preparations on the amounts of neuropeptides measured. Such variation is quite common for commercial sources of *in vivo* tissues. This may be due to different groups of animals, specific conditions for dissection of the fresh tissue by the commercial vendor and transportation.