Pituitary Adenylate Cyclase-Activating Polypeptide and Vasoactive Intestinal Peptide-Stimulated Cyclic AMP Synthesis in Rat Cerebral Cortical Slices

Interaction with Noradrenaline, Adrenaline, and Forskolin

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Abstract

Pituitary adenylate cyclase-activating polypeptide (PACAP; 0.001–1 µM) and vasoactive intestinal peptide (VIP; 0.01–1 µM) produced a concentration-dependent stimulation of cyclic AMP (cAMP) formation in rat cerebral cortical slices prelabeled with [3H]adenine. The effects of PACAP 38 and PACAP27 were similar, and more efficacious (at 0.1 and 1 µM) than those of VIP. Adrenaline and noradrenaline (each at 100 µM) also stimulated cAMP formation, with the latter compound being more effective. Combination of PACAP 38, PACAP27 (each at 0.1 µM) and VIP (1 µM) with adrenaline or noradrenaline resulted in most cases in additive effects, with some supraadditive (PACAP27 plus adrenaline) or subadditive (PACAP 38 or VIP plus noradrenaline) fluctuations. In contrast, combination of each of the three peptides with 3 µM forskolin resulted in synergistic effects. These results indicate that in rat cerebral cortex there is no synergism between PACAP or VIP with noradrenaline or adrenaline; however, based on the forskolin data, it seems likely that synergistic effects may take place with VIP or PACAP and other cAMP-stimulating neuroregulators.

Index Entries: PACAP; VIP; noradrenaline; adrenaline; cAMP, rat cerebral cortex.

Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) are biologically active peptides that display a high degree of structural and functional similarities. PACAP exists in two biologically equipotent short and long forms, consisting, respectively, of 27 and 38 amino acid residues, i.e., PACAP 1-27 (PACAP27) and PACAP 1-38 (PACAP38). VIP is a 28 amino acid peptide, whose primary structure shows 68% homology with the short PACAP form. PACAP (both forms) and VIP equipotently interact with VPAC1 and VPAC2 receptors (VIP = PACAP38 ≈ PACAP27). PACAP also interacts with its specific PAC1 receptors, which under physiological conditions are poorly recognized by VIP (PACAP38 = PACAP27 >> VIP) (Harmar et al., 1998; Vaudry et al., 2000).

PACAP, VIP, and their receptors are widely distributed in brain and peripheral tissues, indicating a pleiotropic nature of the peptides’ activity (Rostene, 1984; Gozes and Brenneman, 1989; Arimura, 1998; Gozes et al., 1999; Vaudry et al., 2000). In the central nervous system (CNS), PACAP and VIP are thought to play a neurotransmitter and/or neuromodulator role; one of the most frequently reported biochemical effects of these peptides is their ability to stimulate adenylyl cyclase activity and cAMP accumulation (e.g., Borghi et al., 1979; Etgen and Browning, 1983; Rostene, 1984; Gozes and Brenneman, 1989; Vaudry et al., 2000; Tatsuno et al., 2001).

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Earlier studies have shown that in its biological actions, VIP plays in concert with some other neuroregulators (e.g., Fredholm and Lundberg, 1982; Emami et al., 1983; Magistretti and Schorderet, 1985; Karbon et al., 1986; Durroux et al., 1987; Schaad et al., 1989; Fatatis et al., 1994; Nowak et al., 1998; Pellegrin et al., 1998). Of particular interest is its synergistic interaction with noradrenaline, a phenomenon first observed in slices of cerebral cortex of mouse (Magistretti and Schorderet, 1984, 1985) and rat (Ferron et al., 1985), using, respectively, biochemical and electrophysiological approaches. This functionally important interaction has subsequently been described to occur in rat pineal gland where the two regulators synergistically act to enhance the activity of both the cAMP- and melatonin-generating systems (Yuwiler, 1987; Chick et al., 1988). Recently, Shioda et al. (2000) reported on PACAP-noradrenaline synergistic interplay with respect to calcium signaling in rat hypothalamus. However, it is unknown whether the described interaction of PACAP and noradrenaline does affect the cAMP generating system, and, if so, whether it can be extended to other brain regions of rat, e.g., cerebral cortex. This work was aimed at elucidating this issue.

Materials and Methods

Animals

Experiments were carried out on male albino Wistar rats weighing 180–220 g. The animals were maintained under a 12 h light/12 h dark lighting schedule (lights on between 06.00 and 18.00) with standard food and water available ad libitum. The experiments were carried out in strict accordance with the Polish governmental regulations concerning experiments on animals.

Assay of cAMP Formation

On the day of experiment, the animals were killed by decapitation between 9.00–9.30, brains were removed, and cerebral cortex (without white matter) isolated and processed for the measurement of cAMP generation. In brief, the tissue pieces (consisting of a part of parietal cerebral cortex) were rapidly cross-sliced (0.25 mm) with the aid of a McIlwain tissue chopper and suspended in cold, O₂/CO₂ (95:5)-gassed, glucose containing modified Krebs-Henseleit medium (KHM; mmol/L): 118, NaCl; 5, KCl; 1.3, CaCl₂; 1.2, MgSO₄; 25, NaHCO₃; 11.7, D-glucose; pH 7.4.

The formation of [³H]cyclic AMP in [³H]adenine-prelabeled tissues was assayed according to Shimizu et al. (1969). The formed [³H]cyclic AMP was isolated by sequential Dowex-alumina column chromatography according to Salomon et al. (1974). The results were individually corrected for a percentage recovery with the aid of [¹⁴C]cyclic AMP added to each column system prior to the nucleotide extraction. The accumulation of cyclic AMP during a 10-min stimulation period was assessed as a percentage of the conversion of [³H]adenine to [³H]cyclic AMP. Details of the whole procedure were described by us earlier (Nowak and Sek, 1994).

Chemicals

The following drugs were used: (±)-adrenaline hydrochloride, forskolin, L-noradrenaline (arterenol) bitartrate, forskolin, PACAP₃₈ (Sigma, St. Louis, MO), PACAP₂₇ (RBI, Natick, MA). Radioactive compounds were: (2,8-[³H]adenine (specific activity 26.9 Ci/mmol) and [¹⁴C]cyclic AMP (specific activity 52.3 mCi/mmol), both from DuPont-NEN (Bad Homburg, Germany).

Data Analysis

All data are expressed as mean ± SEM values. For statistical evaluation of results, analysis of variance (ANOVA) was used followed by the Newman-Keuls test.

Results

As shown in Fig. 1, PACAP₂₇, PACAP₃₈ (0.001–1 µM) and VIP (0.01–1 µM) produced a concentration-dependent stimulation of cAMP formation in rat cerebral cortex. Both forms of PACAP were equipotent, whereas VIP appeared to be less effective than PACAP, especially when used at higher concentrations. The data expressed as “net increases,” i.e., differences between peptide-stimulated values and respective basal values, were (in percent conversion): at 0.1 µM – 5.11 ± 0.92 (17) for VIP, 8.00 ± 0.55 (18) for PACAP₂₇, and 8.20 ± 1.23 (10) for PACAP₃₈.

The study of an interaction between the three peptides and noradrenaline or adrenaline on cAMP formation was carried out using selected concentrations of the compounds, i.e., 0.1 µM for both forms of PACAP, 1 µM for VIP, and 100 µM for catecholamines. At the concentration used, the two catecholamines clearly stimulated cAMP production, however, the effects evoked by adrenaline were always smaller: 0.68 vs 2.89, 0.44 vs 1.24, and 1.52 vs 4.08% conversion (data represent mean values showing net