Stimulation of Cyclic AMP Formation by Pituitary Adenylate Cyclase-Activating Polypeptide Is Attenuated by Glutamate in Rat Brain Slices

Kaoru Kondo, Hitoshi Hashimoto, Kazuko Sakata, Hiroshi Saga, Jun-ichi Kitanaka and Akemichi Baba*

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565, Japan

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ABSTRACT—In rat hippocampal slices, pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38) stimulated cyclic AMP formation in dose- and time-dependent manners. The PACAP-38 action was dose-dependently attenuated by l-glutamate in the hippocampus; l-glutamate at the concentration of 1 mM attenuated PACAP-38-stimulated cyclic AMP formation by approximately 30%. The inhibitory effect of l-glutamate is also observed in rat cerebellar slices. In contrast, the inhibitory effect of a prostanoid EP3-receptor agonist on PACAP-38-stimulated cyclic AMP formation was brain region-specific; the inhibitory action was observed in the cerebellum but not in the hippocampus.

Keywords: Pituitary adenylate cyclase-activating polypeptide, Glutamate, Brain slice

Pituitary adenylate cyclase-activating polypeptide (PACAP), a polypeptide hormone related to vasoactive intestinal polypeptide (VIP), stimulates adenylate cyclase in pituitary cells (1). Two forms of PACAP, PACAP-38 and PACAP-27, are equally potent in stimulating the adenylate cyclase in pituitary cells and rat astrocytes (2, 3). PACAP is present not only in peripheral tissues but also in various regions of the central nervous system (CNS) including the hypothalamus, posterior pituitary, cerebral cortex and hippocampus (4). The cellular actions of PACAP are mediated by specific receptors that are positively coupled to adenylate cyclase on the target cells (1). Two types of PACAP receptors, type I and type II bind PACAP with high affinity; and the type II receptor also binds VIP (1). Previously, several groups including us reported the molecular cloning of these two receptors (3, 5). Northern hybridization and in situ hybridization indicated abundant expression of PACAP receptors in the CNS, especially in the olfactory bulb, granule cell layers of the hippocampal dentate gyrus and glial cells in the cerebellum (ref.3 and authors' unpublished observation).

To assess the function of the PACAP receptor, it is important to find possible regulations of its signal cascade by other receptor systems in each region of the brain. In the present communication, we indicated that PACAP-38 stimulated cyclic AMP formation in rat hippocampal and cerebellar slices and that l-glutamate regulated the action of PACAP-38. In the experiments, we also used a specific agonist for the EP3 subtype of prostanoid receptor that negatively coupled to adenylate cyclase as a reference stimulant (6).

Wistar rats (4–6 weeks, weighing 100–150 g) were decapitated, and their brains were rapidly removed. Brain slices were prepared according to the method reported elsewhere (7). The slices were washed three times with 10 ml fresh Krebs-Ringer bicarbonate buffer (KRB) containing: 117.9 mM NaCl, 4.72 mM KCl, 2.54 mM CaCl2, 1.18 mM MgSO4, 1.19 mM KH2PO4, 25.0 mM NaHCO3 and 11.1 mM D-glucose, equilibrated with 95% O2/5% CO2. Then they were resuspended in the same volume of fresh buffer and incubated for 60 min at 37°C with continuous gassing and vigorous shaking. The medium was then replaced with 1 mM iso-butylmethylxanthine-KRB, and aliquots of the slice suspension (usually 210 µl) were distributed into individual polypropylene test tubes. After the tubes were preincubated another 20 min at 37°C with gassing and shaking, drug solutions (90 µl) were added and incubated for 60 min. l-Glutamate and M&B28767 (15S-hydroxy-9-oxo-16-phenoxy-α-tetranorprost-13E-enonic acid; a gift from Dr. M.P.L. Caton of Rhone-Poulenc, Ltd., Dagenham, Essex, UK), a selective agonist of the EP3 receptor (6), were added 5 min prior to PACAP-38 (Peptide Institute, Inc., Osaka). Incubations were stopped by addition of 300 µl/tube of cold 10%
trichloroacetic acid. The content of cyclic AMP in the samples was determined by radioimmunoassay with a cyclic AMP [125I] assay kit (Amersham, Tokyo) as described elsewhere (8).

PACAP-38 markedly stimulated cyclic AMP formation in hippocampal slices over a concentration range of 0.01–1 μM in 60 min of incubation (Fig. 1A). PACAP-38 (1 μM) markedly increased cyclic AMP levels for up to 60 min of incubation (Fig. 1B). The concentration of PACAP-38 used in this study was higher than that of PACAP-38 used in cultured cells (3). L-Glutamate (1 mM) attenuated the PACAP-38-stimulated cyclic AMP formation at all incubation times tested (Fig. 1B). The inhibitory effect of L-glutamate was dose-dependent in the hippocampus (Fig. 1C). In our preliminary experiment, significant inhibition of PACAP-action was observed at 1 mM L-glutamate (data not shown).

PACAP-38 (1 μM) also stimulated cyclic AMP formation in cerebellar slices by 51% compared to that in hippocampal slices (Fig. 2). L-Glutamate (1 mM) itself had no effect on basal cyclic AMP formation in both hippocampal and cerebellar slices (Fig. 2). The inhibitory effect of L-glutamate on the PACAP-38 action was also observed in cerebellar slices, similarly in the hippocampus (Fig. 2). M&B28767, a selective agonist of the EP3 subtype of prostanoid receptor that is negatively coupled to adenylate cyclase (6), attenuated PACAP-38-stimulated cyclic AMP formation in cerebellar slices. However, it was not the case in the hippocampus; M&B28767 did not inhibit PACAP-38-stimulated cyclic AMP formation in hippocampal slices (Fig. 2). Thus, we suggest that the cyclic AMP response by PACAP-38 is modulated by L-glutamate in the hippocampus and cerebellum. In addition, an agonist specific for the EP3 subtype of prostanoid

Fig. 1. Inhibitory effect of L-glutamate on PACAP-38-stimulated cyclic AMP formation in rat hippocampal slices. A: Dose-dependent curve of PACAP-38-stimulated cyclic AMP formation. B: Time course of PACAP-38 (1 μM)-stimulated cyclic AMP formation in the absence (open circles) or presence (closed circles) of 1 mM L-glutamate. C: Dose-response curves for inhibition of PACAP-38-stimulated cyclic AMP formation by L-glutamate in rat hippocampal slices. Intracellular cyclic AMP levels in slices treated with or without 1 μM PACAP-38 were 658 ± 30 (used as a control value) and 9.7 ± 1.6 pmol/mg protein, respectively. Slices were incubated for 60 min (A, C) or for the indicated periods (B). Values are means ± S.E.M., n=3–10.

Fig. 2. Effects of L-glutamate and M&B28767 on PACAP-38-stimulated cyclic AMP formation in rat hippocampal and cerebellar slices. L-Glutamate (1 mM) and M&B28767 (1 μM) were added with or without PACAP-38 (1 μM) for 60 min. Values are means ± S.E.M. n = 3–6. *P < 0.05, **P < 0.01 vs PACAP-38 (1 μM) by Student’s t-test.
receptor modulates the PACAP-38 action only in the cerebellum. In another set of experiments, we detected the expression of the messenger RNA for the prostanoid EP₃ receptor in both rat hippocampus and cerebellum by the reverse transcriptase-polymerase chain reaction (unpublished data). Those observations as well as the present result (Fig. 2) suggest that in the hippocampus, prostanoid EP₃ receptor-expressing cells have no receptor for PACAP or that hippocampal EP₃ receptor-mediated signal transduction does not cross-react functionally with PACAP receptor-mediated signal transduction. Similar modulatory actions of neuroactive agents such as noradrenaline, histamine and excitatory amino acids on VIP-stimulated cyclic AMP formation were reported by Schaad et al. (9, 10).

In the hippocampus, glutamate neurons are main excitatory inputs and two subtypes of PACAP receptors, type I and type II receptors, are abundantly expressed in the neuronal cells of this region (3). In addition, glutamate and 1S,3R-ACPD, agonists of metabotropic glutamate receptors (mGluRs), attenuated the forskolin-stimulated cyclic AMP production in hippocampal slices (11). We also detected that 1S,3R-ACPD inhibited dose-dependently PACAP-38 (1 µM)-stimulated cyclic AMP formation at concentrations up to 10 µM in hippocampal slices (data not shown). In the cerebellum, in situ hybridization revealed a distribution of mGluR3 (12) and PACAP receptors in glial cells (authors' unpublished observation). mGluR3 is known to be coupled to an adenylate cyclase inhibitory system (13). In other experiments, we found that t-glutamate and M&B28767 attenuated forskolin-stimulated cyclic AMP formation in cultured astrocytes (ref. 8 and authors' unpublished observation). Thus, the present results suggest the co-existence of PACAP receptors and mGluR in neurons of the hippocampus and glial cells of the cerebellum.

In conclusion, results presented in this communication delineate the relevance of neurotransmitter interactions in the modulation of PACAP-neural activity in the CNS.

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