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Research report

VIP enhances synaptic transmission to hippocampal CA1 pyramidal cells through activation of both $VPAC_1$ and $VPAC_2$ receptors

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Abstract

We previously described that vasoactive intestinal peptide (VIP) increases synaptic transmission to hippocampal CA1 pyramidal cells at concentrations known to activate VIP-selective receptors ($VPAC_1$ and $VPAC_2$) but not the PACAP-selective PAC_1 receptor. We now investigated the involvement of $VPAC_1$ and $VPAC_2$ receptors in the effects elicited by VIP as well as the transduction pathways activated by VIP to cause enhancement of synaptic transmission. Blockade of either $VPAC_1$ or $VPAC_2$ receptors with PG 97–269 (100 nM) or PG 99–465 (100 nM) inhibited VIP-induced enhancement of synaptic transmission. Selective activation of $VPAC_1$ receptors with [K^{15}, R^{16}, L^{27}] VIP(1–7)/GRF(8–27) (10 nM) or of $VPAC_2$ receptors with RO 25–1553 (10 nM) increased synaptic transmission to CA1 pyramidal cells, and this increase was larger when both agonists were applied together. Inhibition of either PKA with H-89 (1 μM) or PKC with GF109203X (1 μM) attenuated the effect of VIP (1 nM). GF109203X (1 μM) abolished the effect of the $VPAC_1$ agonist [K^{15}, R^{16}, L^{27}] VIP(1–7)/GRF(8–27) (10 nM) on hippocampal synaptic transmission but that effect was not changed by H-89 (1 μM). The effect of RO 25–1553 (100 nM) obtained in the presence of both the PAC_1 and $VPAC_1$ antagonists, M65 (30 nM) and PG 97–269 (100 nM), was strongly inhibited by H-89 (1 μM) but not GF109203X (1 μM). It is concluded that VIP enhances synaptic transmission to CA1 pyramidal cell dendrites through $VPAC_1$ and $VPAC_2$ receptor activation. $VPAC_1$-mediated actions are dependent on PKC activity, and $VPAC_2$-mediated actions are responsible for the PKA-dependent actions of VIP on CA1 hippocampal transmission.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: Peptides: anatomy and physiology

Keywords: $VPAC_1$; $VPAC_2$; PKA; PKC; Hippocampal slices; fEPSPs

1. Introduction

Vasoactive intestinal peptide (VIP) increases synaptic transmission to hippocampal CA1 pyramidal cells [15]. We recently showed that this excitatory action of VIP involves enhanced inhibition of GABAergic interneurones that control pyramidal cells, thereby promoting their disinhibition [8]. This action occurs both through presynaptic enhancement of GABA release [8,30] and postsynaptic facilitation of GABAergic currents in interneurones [8]. The nature of VIP receptors involved was, so far, not investigated.

VIP acts through activation of two selective high affinity VIP receptors: $VPAC_1$ and $VPAC_2$ receptors that belong to the class II family of G-protein-coupled receptors. These receptors are encoded by two different genes sharing only 55% similarity, have similar affinities for VIP and are positively coupled to adenylate cyclase by G_s activation (see [18] and [27] for review). The VIP receptor family also includes a third receptor, PAC_1, which binds VIP with low affinity [18,27]. The three receptors also recognize with high affinity pituitary adenylate

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cyclase activating peptide (PACAP). $VPAC_1$ and $VPAC_2$ receptors have been identified in the hippocampus by in situ hybridization, autoradiography and immunohistochemistry ([17], see [27] for review). Postsynaptic excitatory actions of VIP on the hippocampal pyramidal cells have been shown to involve either cAMP or cAMP-dependent mechanisms [5,15,16]. However, $VPAC_1$ and $VPAC_2$ receptors can also couple to other signaling/G-protein-dependent mechanisms in different brain preparations [10,14,22], and it is possible that this occurs also in the hippocampus since Shreeve [24] reported that $VPAC_1$ receptor can couple to $G_{i/o}$ protein in this brain region.

The present work was designed to investigate the type of VIP receptor(s) that mediate the excitatory action of VIP on synaptic transmission in the hippocampus, as well as the signaling mechanisms involved in this action of VIP. $VPAC_1$ and $VPAC_2$ agonists and antagonists were used, as well as inhibitors of protein kinase A (PKA) and protein kinase C (PKC). Preliminary accounts of some of the results already appeared [7,9].

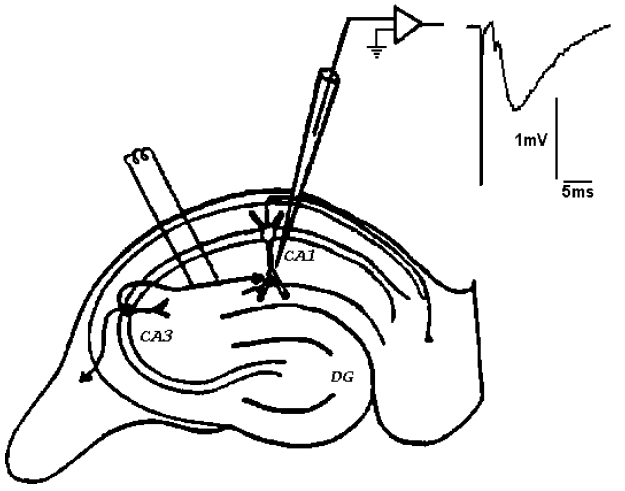

Fig. 1. Recording of fEPSPs in the CA1 area of hippocampal slices. Schematic representation of a hippocampal transverse slice preparation showing the stimulation of the Schaffer collateral pathway (left), the positioning of recording electrode (right) used to obtain extracellular responses in the CA1 dendritic layer of the stratum radiatum and the representative recording of an evoked fEPSP taken from a young adult rat. The fEPSP trace is the average of eight consecutive individual responses and is preceded by the presynaptic volley and the stimulus artefact.

2. Materials and methods

The experiments were performed on hippocampal slices as previously used in our laboratory (e. g. [8]). Male outbread Wistar rats (5–6 weeks, 125–160 g) were anesthetized by halothane inhalation and decapitated. Animal handling was according to European Union (86/609/EEC) guidelines. Briefly, one hippocampus was dissected free in ice-cold Krebs solution of the following composition (mM): NaCl 124, KCl 3, $MgSO_4$ 1, $CaCl_2$ 1.5, glucose 10, NaH_2PO_4 1.25, $NaHCO_3$ 25, gassed with 95% O_2/5% $C0_2$. Slices (400 μm thick) were cut with a McIlwain tissue chopper perpendicular to the long axis of the hippocampus and allowed to recover for at least 1 h in gassed Krebs solution at room temperature. A slice was then transferred to a 1 ml recording chamber for submerged slices and continuously perfused with gassed Krebs solution kept at 30 °C at a flow rate of 4 ml min^{-1}. The chamber and perfusion system were previously coated with BSA (0.1 mg ml^{-1}) to prevent peptide adhesion. Stimulation (rectangular pulses of 0.1 ms duration applied once every 15 s) was delivered through a bipolar concentric electrode placed on the Schaffer collateral/commissural fibers in the *stratum radiatum* near the CA3/CA1 border (Fig. 1). Evoked field excitatory postsynaptic potentials (fEPSPs, Fig. 1) were recorded through an extracellular microelectrode (4 M NaCl, 2–4 MΩ resistance) placed in the *stratum radiatum* of the CA1 area (Fig. 1). The intensity of the stimulus (90–240 μA) was adjusted to obtain a submaximal fEPSP slope with a minimum population spike contamination and near 50% of the fEPSP slope obtained with supramaximal stimulation. Averages of eight consecutive individual responses were obtained, measured, graphically plotted and recorded for further analysis with a personal computer using the LTP software [3]. Responses were quantified as the slope of the initial phase of the averaged fEPSPs because slope measures are considered a more accurate measure of fEPSP magnitude than the amplitude attributable to possible contamination by the population spike.

Drugs were added to the superfusion solution, and drug effects were calculated as the % change of the averaged fEPSP slope obtained in the six recordings immediately before drug application. Since we observed that, when two consecutive VIP applications were performed, the response to the second was smaller than the response to the first VIP application, in the experiments described here, each slice was submitted to a single 30 min VIP application, and the same procedure was used with the $VPAC_1$ and $VPAC_2$ receptor agonists. When the effect of VIP or other VIP receptor agonists was tested in the presence of other drugs, the agonists were applied only after a stable response to those drugs was observed and never before 30 min of their perfusion. When modulatory drugs caused a considerable increase in the responses, stimulation intensities were readjusted to obtain responses near 50% of maximum of the slope of fEPSPs. Responses to the VIP receptor agonists tested in the presence of other drugs were calculated, taking 0% as the averaged fEPSP slope obtained in the six recordings immediately before application of the agonists.

VIP (Novabiochem, Darmstadt, Germany), [Ac-Tyr^1, D-Phe^2] GRF (1–29) (Tocris Cockson, Avonmouth, UK), PG 97–269, PG 99–465, [K^{15}, R^{16}, L^{27}] VIP(1–7)/GRF(8–27) and RO 25–1553 (all four were a kind gift from Prof. Patrick Robberecht, ULB, Brussels, Belgium) were made up in 0.1 mM stock solution in CH_3COOH 1% (v v^{-1}). M65 (a kind gift of Prof. Ethan Lerner, CBRC, MGA,