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Brain Research 1049 (2005) 52 – 60
BRAIN RESEARCH
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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

Diana Cunha-Reis*, Joaquim Alexandre Ribeiro, Ana M. Sebastião

Institute of Pharmacology and Neurosciences, Faculty of Medicine and Institute of Molecular Medicine, University of Lisbon, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal

Accepted 26 April 2005
Available online 3 June 2005

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

* Corresponding author. Fax: +351 217999454.
E-mail address: diana-reis@fm.ul.pt (D. Cunha-Reis).

0006-8993/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.brainres.2005.04.077

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].

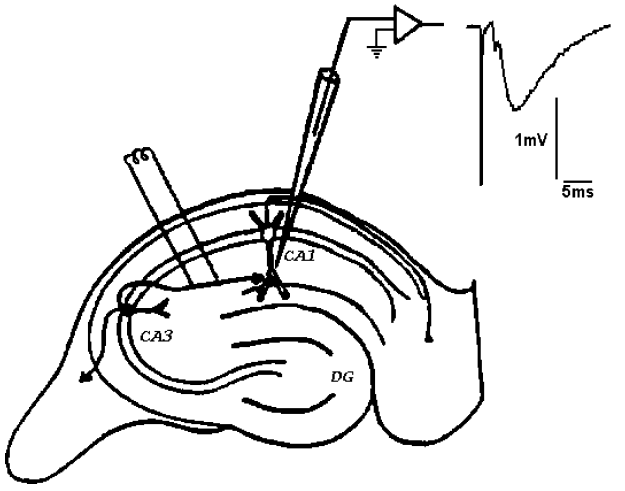

Fig. 1. Recording of fEPSPs in the CA1 area of hippocampal slices. Schematic representation of a hippocampal transverse slice preparation showing the stimulation of the Schaffer collateral pathway (left), the positioning of recording electrode (right) used to obtain extracellular responses in the CA1 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,

MA, USA) was made in 0.1 mM stock solution in PBS. HA1004, H-89, chelerythrine and GF109203X (Sigma-Aldrich, Sintra, Portugal) were made up in 5 mM stock solution in DMSO. The maximal DMSO and CH_3COOH concentrations added to the slices, 0.02 and 0.001% (v v^{-1}), respectively, were devoid of effects on fEPSP slope ($n = 3$). Aliquots of the stock solutions were kept frozen at −20 °C until use. In each experiment, one aliquot was thawed and diluted in Krebs solution.

Values are presented as mean ± SEM from n observations, and the significance of the means was calculated by the Student's t test. When comparing the effect of VIP in more than two experimental conditions, one way analysis of variance (ANOVA) was used followed by Dunnett's multiple comparison test. P values of 0.05 or less were considered to represent significant differences.

3. Results

3.1. VIP activates both $VPAC_1$ and $VPAC_2$ receptors to increase synaptic transmission to CA1 pyramidal cells

We have previously described that VIP causes a biphasic increase in synaptic transmission to pyramidal cell dendrites, generating a bell-shaped concentration–response curve for the facilitation of fEPSP slope with a maximum increase for 1 nM VIP [8]. In this new set of experiments, we used 1 nM VIP which caused a consistent and reversible increase in the slope (21.2 ± 0.7%, $n = 7$, $P < 0.05$, Fig. 2) of the fEPSPs recorded in the CA1 area of the hippocampus.

The involvement of each of the VIP-selective receptors in the effect of VIP on hippocampal synaptic transmission was tested using the $VPAC_1$-selective antagonist PG 97–269 [11] and the $VPAC_2$-selective antagonist PG 99–465

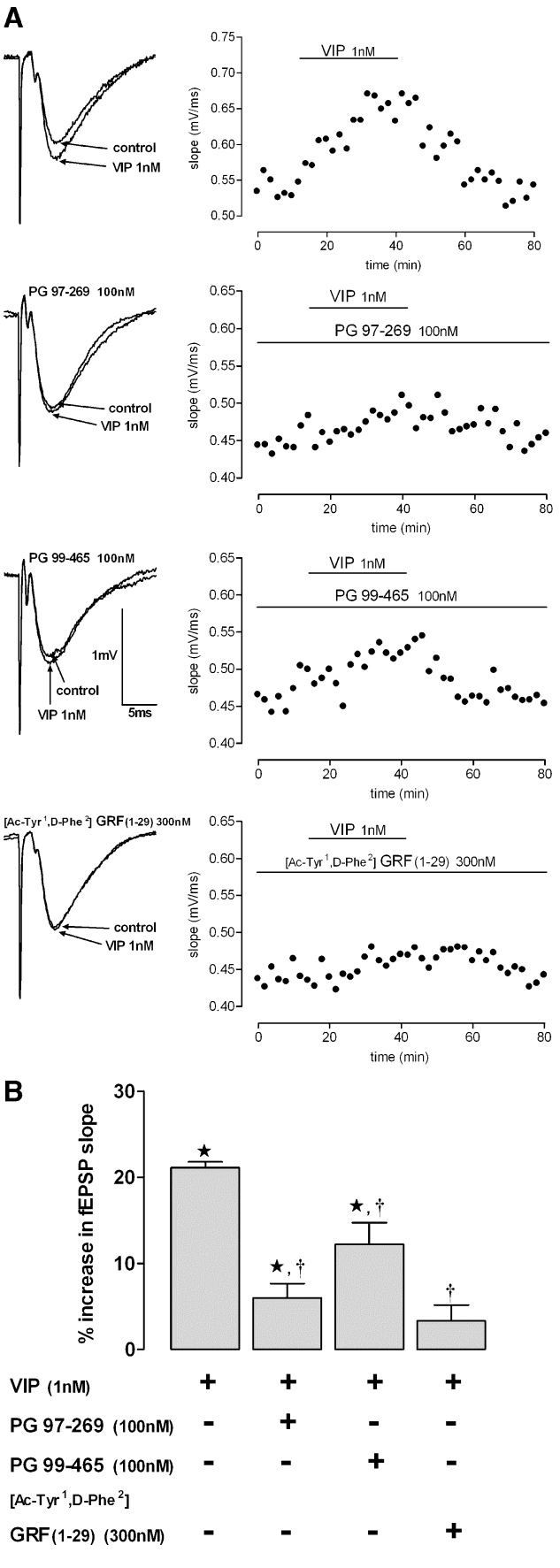

Fig. 2. VIP enhancement of synaptic transmission to CA1 pyramidal cell dendrites is dependent on both $VPAC_1$ and $VPAC_2$ receptor activation. (A) Recordings of field excitatory postsynaptic potentials (fEPSPs, left panels) and time-course of changes in fEPSP slope (right panels) obtained in individual experiments to evaluate the action of VIP either (from top to bottom) applied alone, in the presence of the $VPAC_1$ receptor antagonist PG 97–269 (100 nM), in the presence of $VPAC_2$ receptor antagonist PG 99–465 (100 nM) or in the presence of VIP receptor antagonist [Ac-Tyr^1, D-Phe^2] GRF(1–29) (300 nM, selective for $VPAC_1$ and $VPAC_2$ vs. PAC_1 receptors). fEPSPs obtained in the presence and in the absence of 1 nM VIP are superimposed and were recorded in the stratum radiatum as described in Materials and methods. Each trace is composed of the stimulus artefact followed by the presynaptic volley and the fEPSP and is the average of eight consecutive responses obtained in one typical experiment. VIP (1 nM) was added to the slices at the time indicated by the horizontal bars. (B) The ability of the selective $VPAC_1$ antagonist PG97–269, of the selective $VPAC_2$ antagonist PG 99–465 and of the VIP receptor antagonist [Ac-Tyr^1, D-Phe^2] GRF (1–29) to inhibit the enhancement of fEPSP slope caused by 1 nM VIP is shown. Each column represents the mean ± SEM of results obtained in 4–7 experiments. ★$P < 0.05$ (Student's t test) as compared with 0%. †$P < 0.05$ (ANOVA, followed by Dunnett's multiple comparison test) as compared with the effect of 1 nM VIP in the absence of other drugs (first column).

[21]. In the presence of the $VPAC_1$ receptor antagonist, PG 97–269 (100 nM), the excitatory effect of VIP (1 nM) was nearly abolished (% increase in fEPSP slope caused by VIP was 6.0 ± 1.6, $P < 0.05$, $n = 4$, Fig. 2). When the $VPAC_2$ receptor antagonist PG 99–465 (100 nM) was present, the effect of 1 nM VIP was attenuated, now increasing the fEPSP slope by only 12.3 ± 2.5% ($P < 0.05$, $n = 4$, Fig. 2). To exclude the involvement of pathways other than $VPAC_1$ and $VPAC_2$ receptor activation in VIP enhancement of synaptic transmission, we used [Ac-Tyr^1, D-Phe^2] GRF (1–29) (300 nM), a peptide that can selectively block VIP receptors [29] but not other receptors of the family present in the hippocampus. The effect of VIP (1 nM) on fEPSP slope was abolished ($P > 0.05$, $n = 5$, Fig. 2) in the presence of a supramaximal concentration (300 nM) [20] of this antagonist. In most experiments, and for reasons of time of the protocol, the VIP receptor antagonists were added to the slices in an early stage of slice setup which did not allow the quantification of the effects on fEPSP slope. In experiments where the effects were quantified [Ac-Tyr^1, D-Phe^2], GRF (1–29) (300 nM, $n = 3$) caused no appreciable change on fEPSP slope, PG 97–269 (100 nM) enhanced fEPSP slope by 8.6 ± 3.4%, ($n = 4$) and PG 99–465 (100 nM) increased fEPSP slope by 13.8 ± 2.6%, ($n = 2$). An enhancement of synaptic transmission by the $VPAC_2$ antagonist was not surprising since this compound is a partial agonist of $VPAC_1$ receptors [21].

To confirm the involvement of both VIP receptors in the facilitation of synaptic transmission to pyramidal cell dendrites in the CA1 area of the hippocampus, we tested the action of the $VPAC_1$-selective agonist [K^{15}, R^{16}, L^{27}] VIP(1–7)/GRF(8–27) [12] and the $VPAC_2$-selective agonist RO 25–1553 [13] on fEPSPs. [K^{15}, R^{16}, L^{27}] VIP(1–7)/GRF(8–27) was used in a concentration (10 nM) that is ten times the EC_{50} for stimulation of cAMP production through rat $VPAC_1$ receptors expressed in CHO cells [12] and, as shown in Fig. 3, it increased the fEPSP slope by 14.1 ± 0.4% ($P < 0.05$, $n = 6$). RO 25–1553 was used in a concentration (10 nM) that is ten times its IC_{50} for VIP binding to rat $VPAC_2$ receptors expressed in CHO cells [28] and that binds negligibly to rat $VPAC_1$ [28] and rat PAC_1 receptors [13]. RO 25–1553 (10 nM) increased the fEPSP slope by 5.8 ± 0.6% ($P < 0.05$, $n = 4$, Fig. 3). As shown also in Fig. 3, the agonists of $VPAC_1$ and $VPAC_2$ receptors at the concentrations tested had nearly additive effects, increasing fEPSP slope by 19.9 ± 1.2% ($P < 0.05$, $n = 4$), an enhancement similar to that obtained with 1 nM VIP. A higher concentration of the $VPAC_2$-selective agonist (100 nM, causing nearly maximal activation of rat $VPAC_2$ receptors in the rat gastric fundus [23]) was tested in the presence of the $VPAC_1$ receptor antagonist (PG 97–269, 100 nM) [11] and the PAC_1 receptor antagonist (M65, 30 nM) [26] to avoid loss of selectivity at $VPAC_2$ receptors.

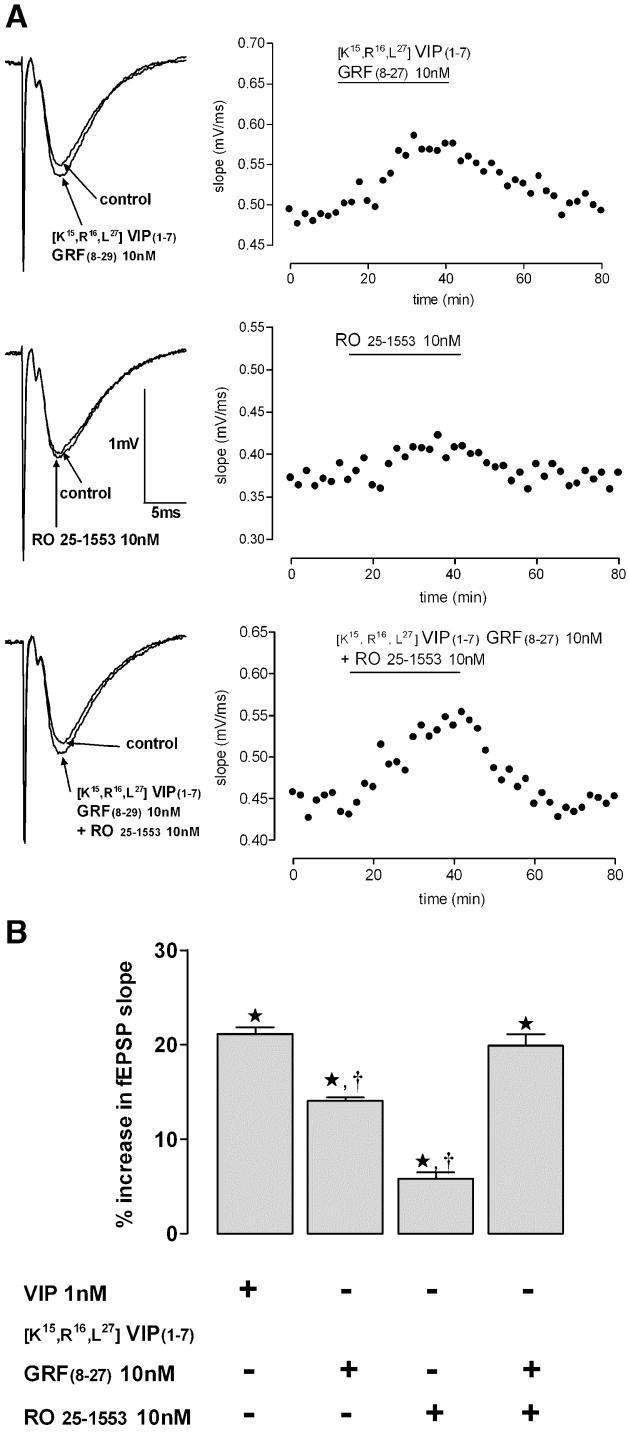

Fig. 3. $VPAC_1$ and $VPAC_2$ receptor activation enhances synaptic transmission to CA1 pyramidal cell dendrites. (A) Recordings of field excitatory postsynaptic potentials (fEPSPs, left panels) and time-course of changes in fEPSP slope (right panels) recorded in individual experiments to evaluate the action of the $VPAC_1$ receptor agonist [K^{15}, R^{16}, L^{27}] VIP(1–7)/GRF(8–27) (10 nM, top), of the $VPAC_2$ receptor agonist RO 25–1553 (10 nM, middle) or both applied together (bottom). fEPSPs obtained in the presence and in the absence of the agonists are superimposed. Selective agonists were added to the slices at the time indicated by the horizontal bars. (B) Averaged effects of the selective VIP receptor agonists upon fEPSP slope. Each column represents the mean ± SEM of results obtained in 3–7 experiments. ★$P < 0.05$ (Student's t test) as compared with 0%. †$P < 0.05$ (ANOVA, followed by Dunnett's multiple comparison test) as compared with the effect of 1 nM VIP (first column).

Under these conditions, the $VPAC_2$ receptor agonist caused a larger (9.8 ± 1.5%, $P < 0.05$, $n = 3$, Fig. 6) enhancement of the fEPSP slope.

3.2. VIP facilitates synaptic transmission to CA1 pyramidal cells through PKA- and PKC-dependent mechanisms

To know if VIP facilitation of fEPSP slope was dependent on PKA activity, we tested VIP effect in the presence of the PKA inhibitors, HA1004 and H-89. When HA1004 (10 μM) was present in the bath, the effect of VIP was smaller (12.3 ± 1.4%, $n = 3$, $P < 0.05$, Fig. 4). Since the concentration of HA1004 could not be increased without loosing selectivity for PKA inhibition [19], we also used H-89, a more selective PKA inhibitor [4]. In the presence of H-89 (1 μM), the excitatory effect of VIP on fEPSP slope was markedly attenuated ($P < 0.05$), with VIP causing only a small though significant increase (9.0 ± 0.7%, $n = 4$, $P < 0.05$, Fig. 4) of fEPSP slope. When applied to hippocampal slices, HA1004 (10 μM) caused no significant ($P > 0.05$, $n = 3$) change in the fEPSP slope, and H-89 (1 μM) caused a slight decrease (−6.6 ± 0.5%, $n = 4$, $P < 0.05$) in the fEPSP slope.

Some VIP-mediated actions in the nervous system have been associated to an increase in PLC activity [22], to the recruitment of intracellular calcium [10] or to an increase in PKC activity [14]. Since PKA inhibitors were not able to block the excitatory action of VIP, we tested whether the facilitation of synaptic transmission by VIP in the CA1 area of the hippocampus could also depend on PKC activity. Upon inhibition of PKC with chelerythrine (6 μM) or the more selective PKC inhibitor, GF 109203X (1 μM) [25], the excitatory effect of 1 nM VIP on fEPSP slope was strongly reduced to 8.5 ± 1.0% ($n = 4$, $P < 0.05$) and to 8.3 ± 2.6% ($n = 4$, $P < 0.05$) (Fig. 4), respectively. When applied to hippocampal slices, chelerythrine (6 μM) and GF 109203X (1 μM) caused a decrease of 12.5 ± 3.5% ($n = 4$, $P < 0.05$) and 12.0 ± 1.7%, ($n = 4$, $P < 0.05$) on the fEPSP slope, respectively.

As shown in Fig. 4, simultaneous PKA and PKC inhibition with H-89 (1 μM) together with GF 109203X (1 μM) abolished the excitatory action of 1 nM VIP on fEPSP slope ($n = 3$, $P > 0.05$). When applied together to hippocampal slices, H-89 (1 μM) and GF-109203X (1 μM) caused a decrease of 21.5 ± 3.6% ($n = 3$, $P < 0.05$) in the fEPSP slope.

To understand which transduction pathways are coupled to the $VPAC_1$ receptor, we tested the effects of the $VPAC_1$-selective agonist upon blockade of PKA with H-89 or blockade of PKC with GF 109203X. In the presence of H-89 (1 μM), the $VPAC_1$ receptor agonist [K^{15}, R^{16}, L^{27}] VIP(1–7)/GRF(8–27) (10 nM) increased fEPSP slope by 13.8 ± 1.3% ($n = 4$, $P < 0.05$, Fig. 5), which is not significantly different ($P > 0.05$) from the effect obtained in the absence of H-89. Use of higher H-89 concentrations was not considered since inhibition of PKC could occur [4];

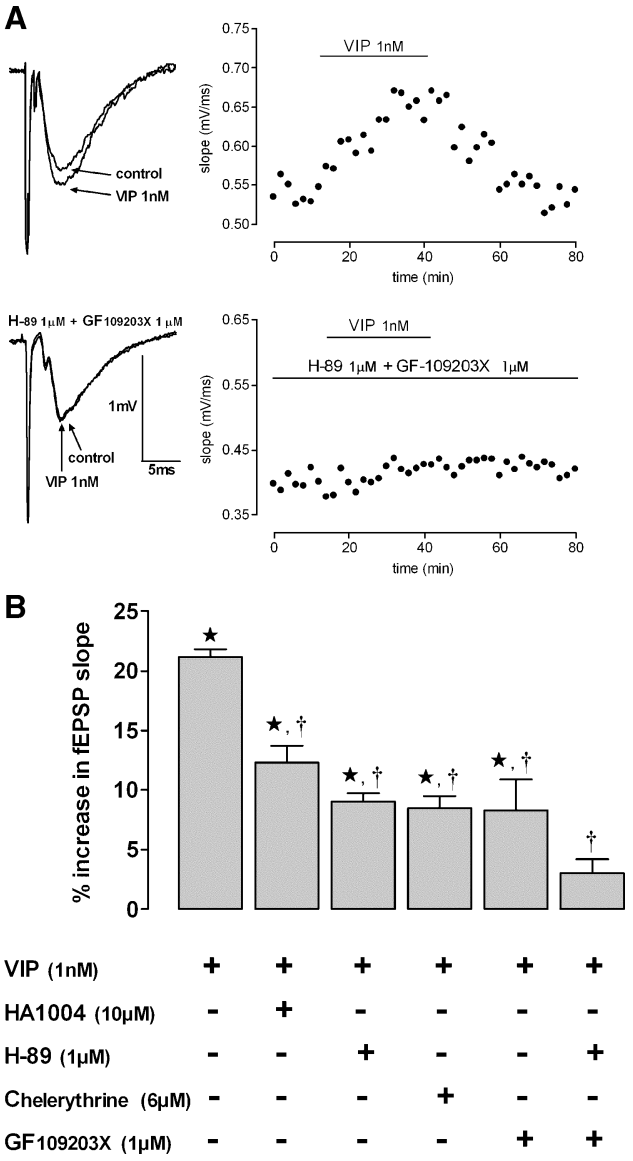

Fig. 4. VIP increases synaptic transmission to CA1 pyramidal cell dendrites through PKA- and PKC-dependent pathways. (A) Recordings of field excitatory postsynaptic potentials (fEPSPs, left panels) and time-course of changes in fEPSP slope (right panels) recorded in individual experiments to evaluate the action of the VIP (1 nM), either (from top to bottom) applied alone or in the presence of both H-89 and GF 109203X. fEPSPs obtained in the presence and in the absence of VIP are superimposed. VIP was added to the slices at the time indicated by the horizontal bars. (B) The ability of the PKA inhibitors, HA1004 and H-89, and of the PKC inhibitors, chelerythrine and GF109203X, to inhibit the excitatory effect of VIP (1 nM) on fEPSP slope is depicted. HA1004 (10 μM) caused no significant ($P > 0.05$) change in the fEPSP slope. H-89 (1 μM), chelerythrine (6 μM) and GF 109203X (1 μM) caused a small decrease of 6.6 ± 0.5% ($n = 4$, $P < 0.05$), 12.5 ± 3.5% ($n = 4$, $P < 0.05$) and 12.0 ± 1.7% ($n = 4$, $P < 0.05$) on the fEPSP slope, respectively. Each column represents the mean ± SEM of results obtained in 3–7 experiments. fEPSPs were recorded in the stratum radiatum as described in Materials and methods. ★$P < 0.05$ (Student's t test) as compared with 0%. †$P < 0.05$ (ANOVA, followed by Dunnett's multiple comparison test) as compared with the effect of 1 nM VIP in the absence of other drugs (first column).

furthermore, this concentration has proved efficient to inhibit the effect of VIP on synaptic transmission (Fig. 4). Upon inhibition of PKC with GF 109203X (1 μM), the excitatory effect of [K^{15}, R^{16}, L^{27}] VIP(1–7)/GRF(8–27) (10 nM) on fEPSP slope was abolished (n = 4, P > 0.05, Fig. 5).

The transduction pathways operated by $VPAC_2$ receptors to enhance synaptic transmission were investigated using 100 nM RO 25–1553 in the presence of the antagonists of $VPAC_1$ and PAC_1 receptors PG 97–269 (100 nM) [11] and M65 (30 nM) [26], respectively. Under these conditions, inhibition of PKA with H-89 (1 μM) abolished the excitatory effect of RO 25–1553 (100 nM) on fEPSP slope (n = 3, P > 0.05, Fig. 6). Upon inhibition of PKC with GF 109203X (1 μM), the excitatory effect of RO 25–1553 (100 nM) on fEPSP slope was smaller (6.5 ± 1.3%, n = 3, P < 0.05, Fig. 6), although it did not attain a significant difference from the effect obtained in the absence of the PKC inhibitor.

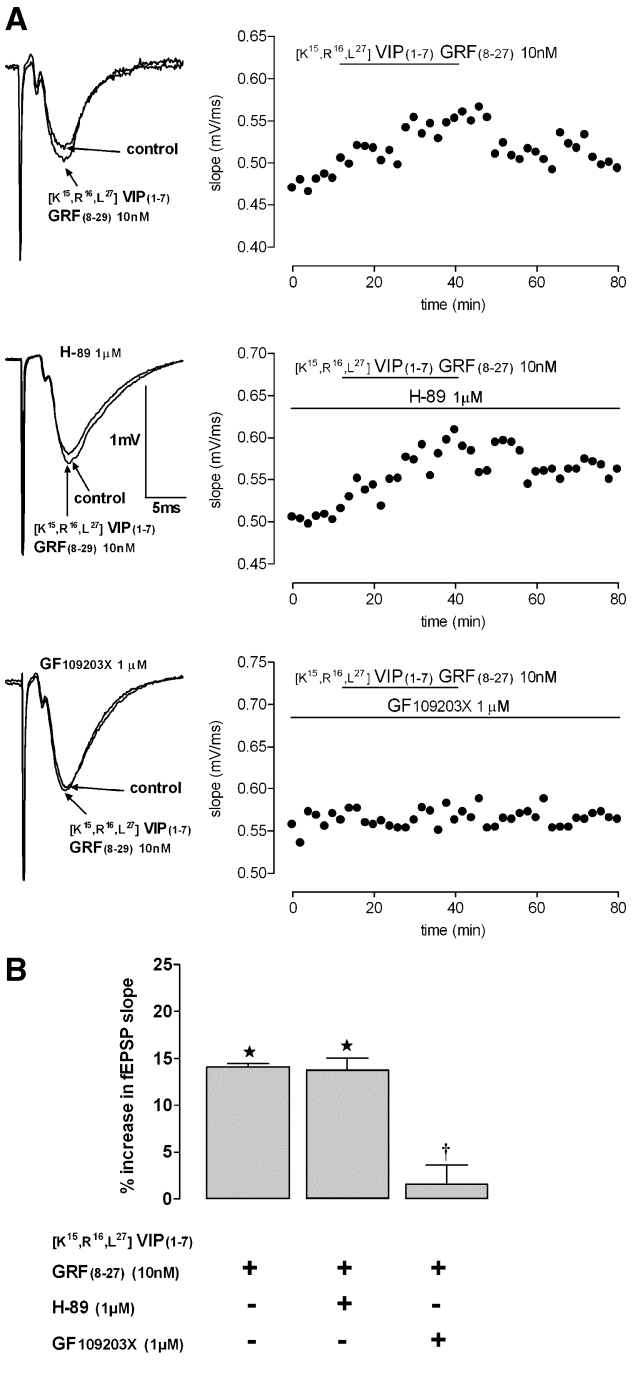

Fig. 5. PKC mediates the enhancement synaptic transmission to CA1 pyramidal cells caused by $VPAC_1$ receptor activation. (A) Recordings of field excitatory postsynaptic potentials (fEPSPs, left panels) and time-course of changes in fEPSP slope (right panels) recorded in individual experiments to evaluate the action of the $VPAC_1$ receptor agonist [K^{15}, R^{16}, L^{27}] VIP(1–7)/GRF(8–27) (10 nM) applied alone (top) in the presence of the PKA inhibitor, H-89 (middle), and of the PKC inhibitor, GF109203X (bottom). fEPSPs were recorded in the stratum radiatum as described in Materials and methods. The $VPAC_1$-selective agonist was added to the slices at the time indicated by the horizontal bars. (B) The ability of the PKA inhibitor H-89 and of the PKC inhibitor GF109203X to inhibit the excitatory effect of [K^{15}, R^{16}, L^{27}] VIP(1–7)/GRF(8–27) (10 nM) on fEPSP slope is depicted. Each column represents the mean ± SEM of results obtained in 6–4 experiments. ★P < 0.05 (Student's t test) as compared with 0%. †P < 0.05 (ANOVA, followed by Dunnett's multiple comparison test) as compared with the effect of 10 nM [K^{15}, R^{16}, L^{27}] VIP(1–7)/GRF(8–27) in the absence of other drugs (first column).

4. Discussion

The main findings in the present work are that (1) VIP activates both $VPAC_1$ and $VPAC_2$ receptors to increase fEPSP slope in the CA1 area of the hippocampus, an increase also observed with selective $VPAC_1$ and $VPAC_2$ receptor agonists; (2) VIP enhancement of fEPSP slope is dependent on both PKA and PKC activities; (3) the increase in fEPSP slope caused by $VPAC_1$ receptor activation is fully dependent on PKC activity; and (4) the enhancement caused by $VPAC_2$ receptor activation is mostly dependent on PKA activity. These observations point towards a functional role for both $VPAC_1$ and $VPAC_2$ receptors in the hippocampus and suggest the existence of more than one signaling mechanism contributing to the enhancement of synaptic transmission to pyramidal cell dendrites by VIP.

This work provides the first evidence for the involvement of both $VPAC_1$ and $VPAC_2$ receptors in the modulation of hippocampal synaptic transmission by VIP. First, because $VPAC_1$ and $VPAC_2$ selective antagonists were able to inhibit the enhancement of synaptic transmission caused by 1 nM VIP, and concomitant blockade of $VPAC_1$ and $VPAC_2$ receptors abolishes VIP action on synaptic transmission. Secondly, because selective activation of $VPAC_1$ and $VPAC_2$ receptors also enhanced hippocampal synaptic transmission and activation of both receptors with these agonists nearly reproduces the effects of VIP. Our data also suggest that VIP enhancement of synaptic transmission is mainly mediated by $VPAC_1$ receptor activation since the effect of the $VPAC_1$-selective agonist is nearly as big as the effect of VIP, and the $VPAC_2$ selective agonist, in a

concentration (100 nM) that is nearly maximal for $VPAC_2$-mediated function in the rat gastric fundus [23], can only cause half the effect of VIP. Furthermore, the effect of VIP in the presence of the $VPAC_1$ receptor antagonist is very small. Fine-mapping of VIP receptors in the hippocampus by immunohistochemistry [17] and also by autoradiography [28] with $VPAC_1$ and $VPAC_2$ selective ligands indicate that the $VPAC_2$ receptor subtype is the most abundant in the Ammon's Horn in the hippocampus and in particular in the pyramidal cell layer, suggesting a major role for this receptor in modulation of hippocampal pyramidal cell activity. $VPAC_1$ receptors in the Ammon's Horn appear to be located in the stratum oriens and radiatum [17,28] and were co localized with glial markers [17]. No markers for interneurones were used in these studies, but it is known that a subpopulation of interneurones that express VIP are specialized to innervate other interneurones in this brain area [2]. If some of these $VPAC_1$ receptors are located in interneurones, this may explain why the presently observed excitatory action of VIP upon synaptic transmission is mainly $VPAC_1$ receptor mediated since this action of VIP results from inhibition of inhibitory circuits directly impinging on pyramidal cell dendrites that are most likely located at the stratum radiatum [2,8].

VIP receptors are coupled to G proteins of the G_s subtype and to adenylate cyclase [18]. However, some VIP-mediated actions are dependent on protein G_s but not on PKA (e.g. [31]). We observed that VIP-mediated enhancement of synaptic transmission is dependent on PKA activity, which is consistent with recent observations that VIP enhances EPSCs in CA1 pyramidal neurones through a PKA-dependent mechanism [5]. In addition, we found that VIP enhancement of CA1 hippocampal synaptic transmission is also dependent on PKC activity since it was attenuated by PKC inhibitors. VIP can activate the phospholipase C/IP3/PKC transduction pathway in several nervous system preparations [10,14,22]. The $VPAC_1$ receptor is coupled to G proteins of the $G_{i/o}$ subtype [24] in the hippocampus, and it is known that $G_{i/o}$ proteins are able to activate PLCβ through Gβγ subunits [6]. Therefore, this is a possible mechanism for the now observed PKC-dependent actions of VIP and of the $VPAC_1$ receptor agonist on hippocampal synaptic transmission. Although PKA and PKC inhibitors could attenuate VIP action on hippocampal synaptic transmission, complete blockade of VIP action could only be achieved with simultaneous blockade of PKA and PKC, implying that VIP enhancement of synaptic transmission requires both the cAMP/PKA and the PLC\IP3\PKC transduction pathways. In addition, the effect of the $VPAC_1$ receptor agonist

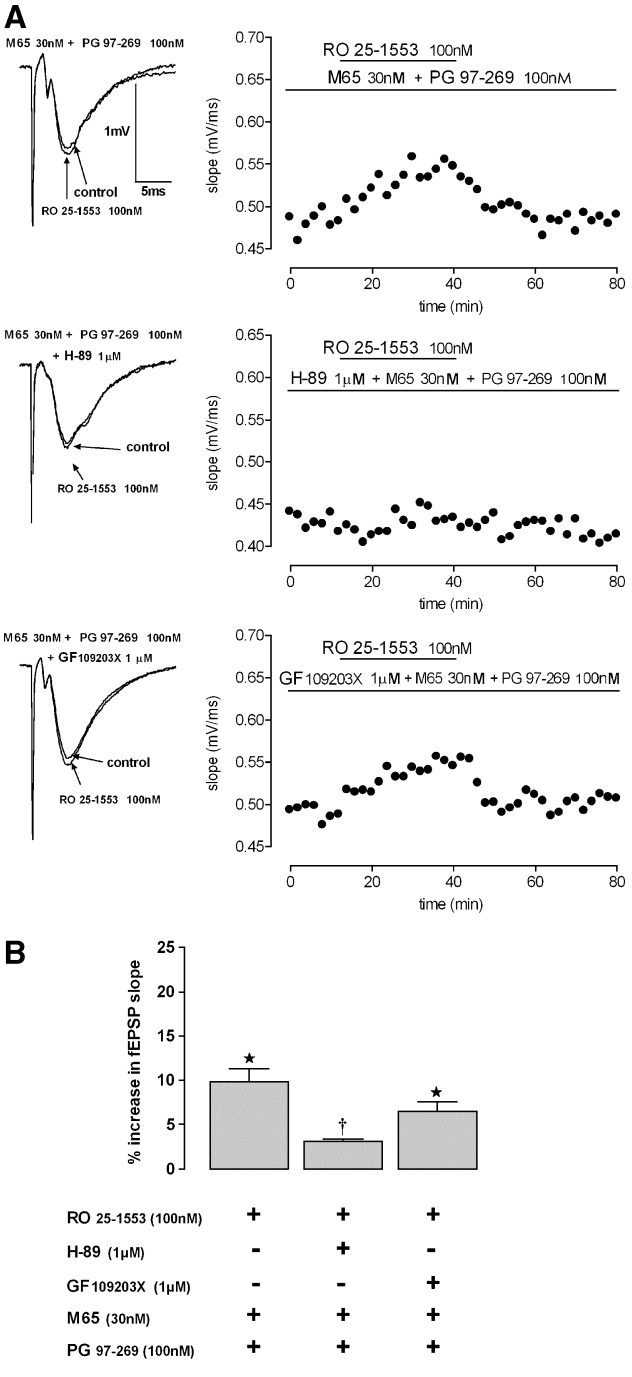

Fig. 6. PKA mediates the enhancement of synaptic transmission to CA1 pyramidal cells caused by $VPAC_2$ receptor activation. (A) Recordings of field excitatory postsynaptic potentials (fEPSPs, left panels) and time-course of changes in fEPSP slope (right panels) recorded in individual experiments to evaluate the action of the $VPAC_2$ receptor agonist RO 25–1553 (100 nM) in the absence (top) or in the presence of the PKA inhibitor, H-89 (middle), or the PKC inhibitor, GF109203X (bottom). The experiments were performed in the presence of the PAC_1 and $VPAC_1$ receptor antagonists M65 (30 nM) and PG 97–269 (100 nM) to avoid loss of selectivity of RO 25–1553 (100 nM). fEPSPs were recorded in the stratum radiatum as described in Materials and methods. The $VPAC_2$ selective agonist was added to the slices at the time indicated by the horizontal bars. (B) The ability of the PKA inhibitor H-89 and of the PKC inhibitor GF109203X to inhibit the excitatory effect of RO 25–1553 (100 nM) on fEPSP slope obtained in the presence of both the PAC_1 and $VPAC_1$ receptor antagonists is depicted. Each column represents the mean ± SEM of results obtained in 3 experiments. ★$P < 0.05$ (Student's t test) as compared with 0%. †$P < 0.05$ (ANOVA, followed by Dunnett's multiple comparison test) as compared with the effect of 100 nM RO 25–1553 in the presence of M65 and PG 97–269 (first column).

was not modified by the PKA inhibitor, suggesting that this receptor operates mainly through a PKA-independent mechanism and that $VPAC_2$ receptor activation is the source for the PKA-dependent actions of VIP.

The fact that two different VIP receptors both lead to an enhancement of synaptic transmission in hippocampal slices may result from the measurement of a final outcome of multiple cellular responses to VIP. VIP is expressed in the hippocampus in three distinct subtypes of interneurones [1,2], selectively targeting either pyramidal cells or interneurones in different hippocampal layers. VIP enhancement of synaptic transmission in the CA1 area of the hippocampus depends on both pre- and postsynaptic modulation of GABAergic transmission [8]. Yet, VIP enhancement of EPSCs in pyramidal cells is observed in the absence of GABAergic transmission [5]. Thus, VIP appears to modulate hippocampal synaptic transmission in a complex manner, involving multiple mechanisms that can influence the response at the integrated level. The uneven cellular and layer distribution of $VPAC_1$ and $VPAC_2$ receptors in the hippocampus may also add to this complexity, and receptors located at different sites in the hippocampus may well be activated by different stimulus occurring in vivo [1,2,17,28]. Further elucidation of the cellular location (dendritic, somatic or nerve terminal) of the different VIP receptors might prove useful to understand the role of each receptor in the control of hippocampal synaptic transmission.

Acknowledgments

We greatly acknowledge Prof. P. Robberecht, SM, ULB, Belgium for the kind gift of VIP selective agonists and antagonists, Prof. Ethan Lerner, CBRC, MGA, MA, USA for the PAC_1 selective ligands and the Institute of Physiology, FML for animal housing facilities. Diana Cunha-Reis was in receipt of an FCT PhD fellowship. This work was supported by FCT.

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