British Journal of Pharmacology (2004) 143, 733–744 © 2004 Nature Publishing Group All rights reserved 0007–1188/04 $30.00
www.nature.com/bjp

VIP enhances both pre- and postsynaptic GABAergic transmission to hippocampal interneurones leading to increased excitatory synaptic transmission to CA1 pyramidal cells

*,[1,2]Diana Cunha-Reis, [1]Ana M. Sebastião, [2]Kerstin Wirkner, [2]Peter Illes & [1]Joaquim Alexandre Ribeiro

[1]Institute of Pharmacology and Neurosciences, Faculty of Medicine and Institute of Molecular Medicine University of Lisbon, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal and [2]Rudolf-Boehm Institute for Pharmacology and Toxicology, University of Leipzig, Haertelstrasse 16-18, D-04107 Leipzig, Germany

1 Vasoactive intestinal peptide (VIP) is present in the hippocampus in three subtypes of GABAergic interneurones, two of which innervate preferentially other interneurones, responsible for pyramidal cell inhibition. We investigated how pre- and postsynaptic modulation of GABAergic transmission (to both pyramidal cells and interneurones) by VIP could influence excitatory synaptic transmission in the CA1 area of the hippocampus.

2 VIP (0.1–100 nM) increased [^{3}H]GABA release from hippocampal synaptosomes (maximum effect at 1 nM VIP; 63.8±4.0%) but did not change [^{3}H]glutamate release.

3 VIP (0.3–30 nM) enhanced synaptic transmission in hippocampal slices (maximum effect at 1 nM VIP; field excitatory postsynaptic potentials (epsp) slope: 23.7±1.1%; population spike amplitude: 20.3±1.7%). The action on field epsp slope was fully dependent on GABAergic transmission since it was absent in the presence of picrotoxin (50 μM) plus CGP55845 (1 μM).

4 VIP (1 nM) did not change paired-pulse facilitation but increased paired-pulse inhibition in CA1 pyramidal cells (16.0±0.9%), reinforcing the involvement of GABAergic transmission in the action of VIP.

5 VIP (1 nM) increased muscimol-evoked inhibitory currents by 36.4±8.7% in eight out of ten CA1 interneurones in the *stratum radiatum*. This suggests that VIP promotes increased inhibition of interneurones that control pyramidal cells, leading to disinhibition of synaptic transmission to pyramidal cell dendrites.

6 In conclusion, concerted pre- and postsynaptic actions of VIP lead to disinhibition of pyramidal cell dendrites causing an enhancement of synaptic transmission.
British Journal of Pharmacology (2004) **143,** 733–744. doi:10.1038/sj.bjp.0705989

Keywords: VIP; GABA; interneurones; hippocampus; rat; slices; patch clamp; synaptosomes

Abbreviations: CCK, cholecystokinin; PPF, paired-pulse facilitation; PPI, paired-pulse inhibition; VIP, vasoactive intestinal peptide

Introduction

Vasoactive intestinal peptide (VIP) is expressed in the hippocampus only in interneurones (Acsády *et al*., 1996a), suggesting that it might be involved in regulation of hippocampal GABAergic transmission. Three distinct subtypes of VIP-immunoreactive interneurones have been described (Acsády *et al*., 1996a, b), having differential target selectivity. One consists of basket cells, responsible for somatic pyramidal cell inhibition, that are also immunoreactive for cholecystokinin (VIP/CCK-immunoreactive basket cells). The two other subtypes innervate selectively interneurones either at the *stratum Oriens/Alveus* border or at the *stratum radiatum*. These target neurones control synaptic transmission to pyramidal cells at distal dendrites in the *stratum lacunosum-moleculare* and proximal dendrites in the *stratum radiatum*, respectively (Acsády *et al*., 1996b). All classes of VIP-immunoreactive interneurones are innervated by serotonergic fibres from the median raphe nucleus (Papp *et al*., 1999) and also by GABAergic septal afferents like virtually all interneurone subtypes in the hippocampus (Freund & Antal, 1988; Papp *et al*., 1999). In addition, VIP/CCK-immunoreactive basket cells receive afferents from CA3 Schaffer collaterals (Acsády *et al*., 1996a) and are avoided by cholinergic septal afferents (Papp *et al*., 1999). VIP-immunoreactive interneurone-selective cells in the CA1 and CA3 areas also appear to receive input from the enthorhinal cortex projection to the stratum lacunosum-moleculare, since their main dendritic arborization is located in this layer (Acsády *et al*., 1996a). VIP acts through activation of two VIP-selective receptors: $VPAC_1$ and $VPAC_2$ receptors (see Harmar *et al*., 1998 for review), both positively coupled to adenylate cyclase. Both

*Author for correspondence at: Institute of Pharmacology and Neurosciences, Faculty of Medicine and Institute of Molecular Medicine University of Lisbon, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal; E-mail: diana-reis@fm.ul.pt
Advance online publication: 25 October 2004

receptor subtypes have been identified in the hippocampus by autoradiography (Vertongen *et al*., 1997).

VIP was shown to increase excitability of hippocampal pyramidal cells essentially through reduction of the Ca^{2+}- and cAMP-dependent K^+ conductance, leading to a decrease of the long-lasting afterhyperpolarization and the accommodation of firing (Haas & Gähwiler, 1992). This action is postsynaptic since it prevailed in low-Ca^{2+} – high-Mg^{2+} medium. VIP was also shown to increase the frequency of miniature ipscs to cultured pyramidal neurones without affecting their amplitude (Wang *et al*., 1997), suggesting a presynaptic action of VIP in the control of GABA release. These actions of VIP seem contradictory since they lead to opposing actions on pyramidal cell excitability. Yanovski *et al*. (1997) studied the influence of VIP application to one subtype of interneurones targeted by VIP-immunoreactive interneurones that are located at the *Oriens/Alveus* border. In the absence of synaptic interactions, VIP (100 nM, in the bath) increased the firing rate of these interneurones. In the same study, local application of VIP (10 μM; pressure applied) to the *Oriens/Alveus* border decreased the slope of field excitatory postsynaptic potentials (epsps) recorded in the *stratum radiatum* and *stratum lacunosum-moleculare*. VIP thus led to a decrease in excitatory synaptic transmission, which is likely due to an increase in inhibitory transmission, and these mechanisms could not account for the previously observed increase in synaptic transmission and pyramidal cell firing (Haas & Gähwiler, 1992).

To understand the role of hippocampal GABAergic interneurones in VIP-mediated facilitation of synaptic transmission, we have now evaluated how pre- and postsynaptic modulation of GABAergic transmission (to both pyramidal cells and interneurones) by VIP could influence synaptic transmission to CA1 pyramidal neurones.

A preliminary account of some of the results already appeared (Cunha-Reis *et al*., 2002b).

Methods

Animals

Male outbread Wistar rats were purchased from Harlan Iberica (Spain) and housed in the local Animal House until use, or were from the Animal House of the Faculty of Medicine of the University of Leipzig (patch-clamp experiments). Animal housing and handing was according to the Portuguese and German laws and European Union (86/609/EEC) guidelines. Rats were 5–7 weeks old for all experiments except for the patch experiments for which 10 to 14-day old animals were used. The animals were anaesthetized by halothane inhalation prior to decapitation.

[^{3}H]GABA release from hippocampal nerve terminals

Release of [^{3}H]GABA from isolated hippocampal nerve terminals (synaptosomes) was measured as in previous experiments from our laboratory (e.g. Cunha *et al*., 1997). Briefly, the purified synaptosomes were loaded for 20 min with [^{3}H]GABA (59.2 kBq ml^{-1}, 18.75 nM) in the presence of unlabelled GABA (0.125 μM), introduced into four parallel 900 μl perfusion chambers ($\approx$0.58 mg_{prot} per chamber) and kept in continuous perfusion (0.6 ml min^{-1}) with oxygenated (95% O_2 and 5% CO_2) Krebs solution (NaCl 125 mM, KCl 3 mM, NaH_2PO_4 1 mM, $NaHCO_3$ 25 mM, $CaCl_2$ 1.5 mM, $MgCl_2$ 1.2 mM, glucose 10 mM) also containing 0.1 mM (aminooxy)acetic acid and 1 μM nipecotic acid, to avoid GABA reuptake by synaptosomes. Because VIP is readily adsorbed onto plastic and glass, the chamber and all the connecting tubes were pretreated with bovine serum albumin (BSA, 0.1 mg ml^{-1}) to minimize losses of the peptide (see Haas & Gähwiler, 1992). After a 30 min equilibration period, the effluent was collected in 2-min fractions ($\approx$1.2 ml) and samples were kept for scintillation counting analysis. In order to determine the amount of tritium retained by synaptosomes, the filters were also kept for scintillation counting. The amount of [^{3}H]GABA in each sample was expressed as a percentage of the total radioactivity present in the preparation at the point of sample collection (fractional release). Synaptosomes were stimulated at 4 min (S_1) and 22 min (S_2) after starting sample collection, with K^+ (28 mM) for 2 min (isomolar substitution of Na^+ by K^+ in the perfusion buffer). Tritium released by stimulation was about 50% Ca^{2+}-dependent (see Results) and mainly composed of [^{3}H]GABA, as shown by HPLC analysis (Cunha *et al*., 1997). In test conditions, VIP was present from the 16th (6 min before S_2) up to the 32nd min after starting sample collection. When testing the action of VIP in the presence of other drugs, these were present from 15 min before starting sample collection until the end of the experiment. The amount of radioactivity released by stimulation was calculated by integration of the area under the evoked peak of tritium release upon subtraction of basal tritium release. Effects (taken as % change) were evaluated by modification of the S_2/S_1 ratios in test and control conditions.

Synaptosomal disruption was evaluated by comparing lactate dehydrogenase activity (Keiding *et al*., 1974) in the incubation bath with that found in the synaptosomal pellet upon its disruption with 2% (v v^{-1}) Triton X-100. The protein content of the hippocampal synaptosomal fraction was determined by the Lowry method modified according to Peterson (1977), using BSA as a standard.

[^{3}H]glutamate release experiments

Release of [^{3}H]glutamate was performed essentially as described above for [^{3}H]GABA with minor modifications. The purified synaptosomes were loaded for 10 min with [^{3}H]glutamate (79.2 mCi ml^{-1}, 1.8 nM) as described by Lonart *et al*. (1996), introduced into four parallel 900 μl perfusion chambers ($\approx$0.32 mg_{prot} per chamber) and kept in continuous perfusion (1.0 ml min^{-1}) with oxygenated (95% O_2 and 5% CO_2) Krebs solution. The perfusion system was previously coated with BSA (0.1 mg ml^{-1}) to prevent VIP adhesion. After a 20 min equilibration period, the effluent was collected in 1-min fractions ($\approx$2.0 ml) and samples were kept for scintillation counting analysis. Synaptosomes were stimulated twice, at 3 min (S_1) and 12 min (S_2) after starting sample collection, with K^+ (20 mM) for 30 s (isomolar substitution of Na^+ by K^+ in the perfusion buffer). In test conditions, VIP was present from the 7th (5 min before S_2) up to the 18th min after starting sample collection. Evaluation of evoked release and drug effects are as described above for [^{3}H]GABA release.

Extracellular recordings in the CA1 area of hippocampal slices

The experiments were performed on hippocampal slices as previously used in our laboratory (e.g. Sebastião *et al*., 2001). The animals were decapitated and one hippocampus 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% CO_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 fibres in the *stratum radiatum* near the CA3/CA1 border. Orthodromically evoked field epsps and population spikes (PSs) were recorded through an extracellular microelectrode (4 M NaCl, 2–4 MΩ resistance) placed in the *stratum radiatum* of the CA1 area or in the *stratum pyramidale* of the CA1 area, respectively. When recording epsps, the intensity of the stimulus (90–240 μA) was adjusted to obtain a submaximal epsp slope with a minimum PS contamination and near 50% of the epsp slope obtained with supramaximal stimulation. When recording PSs, the intensity of the stimulus (200–400 μA) was adjusted to evoke a response with amplitude near 50% of the PS amplitude obtained with supramaximal stimulation. Paired-pulse facilitation (PPF) was evaluated by applying two consecutive stimuli (50 ms interval) once every 15 s and recording field epsps. Paired-pulse inhibition (PPI) was evaluated by applying two consecutive stimuli (10 ms interval, for which maximal PPI was observed) once every 15 s and recording PSs. The average of eight consecutive individual responses were obtained, measured, graphically plotted and recorded for further analysis with a personal computer using the LTP software (Anderson & Collingridge, 1997). Responses were quantified either as the slope of the initial phase of the averaged epsps or as the amplitude of the averaged PSs. PPF and PPI were expressed as the ratio between the averaged responses to the second (S_2) and the first (S_1) stimulation pulses. Drugs were added to the superfusion solution. 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. Therefore, the concentration–response curves showed in this paper were performed in a noncumulative manner. When the effect of VIP was tested in the presence of other drugs, VIP was applied only after a stable response to these drugs was observed and never before a 30 min perfusion of these drugs.

Patch-clamp recordings in the CA1 area of hippocampal slices

Rats were decapitated and the brain was rapidly removed and placed in ice-cold, oxygenated (95% O_2 plus 5% CO_2) Krebs solution (pH 7.4) of the composition (mM) NaCl 126, KCl 2.5, $MgCl_2$ 1.3, $CaCl_2$ 2.4, glucose 11, NaH_2PO_4 1.2, $NaHCO_3$ 25, also containing 1 mM ascorbic acid and 3 mM pyruvic acid. Thin coronal slices (200 μm thick) from hemisected brains containing parts of the cortex and the basal ganglia were cut with a tissue slicer (Vibratome 1000, Plano, Marburg, Germany) and allowed to recover for 1 h in a holding chamber at 37°C. When starting the recordings, single slices were transferred to a recording chamber (300–400 μl volume) and superfused (3 ml min^{-1}) with oxygenated Krebs solution at room temperature (23–25°C).

Membrane currents were recorded from both CA1 pyramidal cells and interneurones in *stratum radiatum* and *stratum lacunosum-moleculare* by procedures similar to those described by Edwards *et al*. (1989) adapted to our experimental conditions (e.g. Wirkner *et al*., 2000). Cells were visualized with an upright interference contrast microscope and a $\times 40$ water immersion objective (Axioscop FS, Carl Zeiss, Oberkochen, Germany). The microscope was connected to a video camera sensitive to infrared light (Newvicon C 2400-07-C, Hamamatsu, Hersching, Germany). Patch pipettes were produced by a micropipette puller (Flaming/Brown P-97, Sutter, Novato, CA, U.S.A.) from borosilicate glass capillaries (outer diameter 2 mm) and filled with intracellular solution of the following composition (mM): K-gluconate 140, NaCl 10, $MgCl_2$ 1, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulphonic acid) (HEPES) 10, ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) 11, MgATP 1.5 and LiGTP 0.3; pH 7.4 adjusted with KOH. Pipette resistances were in the range of 5–8 MΩ. The liquid junction potential (V_{LJ}) between the bath and the pipette solutions at 22°C was calculated according to Barry (1994) and it was found to be 15.2 mV. All membrane potential values given in this study were corrected for V_{LJ}. Cells were clamped at −70 mV, taking into account this potential. The membrane potential of the neurones was measured in the current clamp mode of the patch amplifier (Axopatch 200-B, Axon Instruments, Foster City, CA, U.S.A.) immediately after establishing whole-cell access and was -55.1 ± 1.3 mV ($n = 9$) for pyramidal cells and -64.0 ± 2.3 mV ($n = 11$) for interneurones. Compensation of the membrane capacitance and series resistance, achieved with the inbuilt circuit of the amplifier, was in the range of 17.4–48.3 pF and 10.2–33.6 MΩ for pyramidal cells and 10.0–65.0 pF and 15.5–57.5 MΩ for interneurones, respectively. Data were filtered at 2 kHz with the inbuilt filter of the Axopatch 200-B, digitized at 5 kHz and stored on a laboratory computer using a Digidata 1200 interface and pClamp 6.0 or AxoScope software (Axon Instruments).

Pyramidal cells in the hippocampus were identified visually in the densely packed arrangement of *stratum piramidale* by their large soma (26–32 μm) and characteristic shape. Interneurones in the *stratum radiatum* and *stratum lacunosum-moleculare* were also identified visually, having cell bodies in the 16–24 μm size range, and often a fusiform shape. All cells chosen for recording were neurones, as judged from the firing of action potentials during the establishment of the gigaseal.

Puff applications of muscimol (30 μM) were performed once every 3 min for 2 s, nine times in a run, by local pressurized superfusion using a DAD-12 system (Adam and List, Westbury, NY, U.S.A.). VIP (1 nM) was applied in the bath solution from 2 min before the fourth current to 1 min after the sixth current by means of a three-way tap; about 20 s were required for VIP to reach the bath. VIP (1 nM) was also

coapplied with muscimol puffs during this period. VIP was then washed out from the bath, and muscimol was alone applied until the end. Current amplitudes in each experiment were normalized to the amplitude of the current recorded immediately before VIP application. Whenever VIP enhanced the currents, effects of VIP were calculated by comparing the amplitude of the third current during VIP application with the average between the current immediately before VIP application and the third current after VIP washout (control).

Drugs

VIP (Novabiochem) and [Ac-Tyr^1, D-Phe^2] GRF(1–29) (Tocris Cookson) were made up in 0.1 mM stock solution in CH_3COOH 1% ($v\,v^{-1}$). Picrotoxin (Sigma/RBI) and CGP55845 (((2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl] (phenylmethyl)phosphinic acid), a kind gift from Ciba-Geigy) were made up in 100 and 10 mM stock solution in dimethylsulphoxide. The maximal dimethylsulphoxide and CH_3COOH concentrations used were devoid of effects on synaptic transmission, pyramidal cell excitability, GABAergic currents or radio-labelled GABA and glutamate release. Muscimol, GABA, (aminooxy)acetic acid (AOAA) and (±)-nipecotic acid were obtained from Sigma/RBI and prepared in aqueous stock solution. γ-Amino-*n*-[2,3-^{3}H]butyric acid ([^{3}H]GABA) and L-[G-^{3}H]glutamic acid ([^{3}H]glutamate) were obtained from Amersham. 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.

Analysis of the data

Values are presented as mean ± s.e.m. 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. A *P*-value of 0.05 or less was considered to represent significant differences.

Results

VIP enhanced K^+-evoked [^{3}H]GABA release from isolated nerve terminals

In synaptosomes incubated with [^{3}H]GABA, the amount of tritium retained at the beginning of sample collection was $(5.96 \pm 0.71) \times 10^{10}$ Bq $mg^{-1}_{protein}$ ($n = 133$). The initial basal release of tritium was $(5.08 \pm 0.78) \times 10^7$ Bq $mg^{-1}_{protein}$ ($n = 43$), giving a fractional release of 0.085 ± 0.005% ($n = 43$) for the first sample collected. Stimulation of synaptosomes with 28 mM K^+ for 2 min (S_1) caused a nearly four-fold increase in the amount of tritium released (Figure 1a), which was also observed for the second stimulation pulse (S_2) so that the S_2/S_1 ratio obtained was 0.848 ± 0.008 ($n = 43$). Addition of 0.5 mM EGTA and reduction of the extracellular calcium concentra-

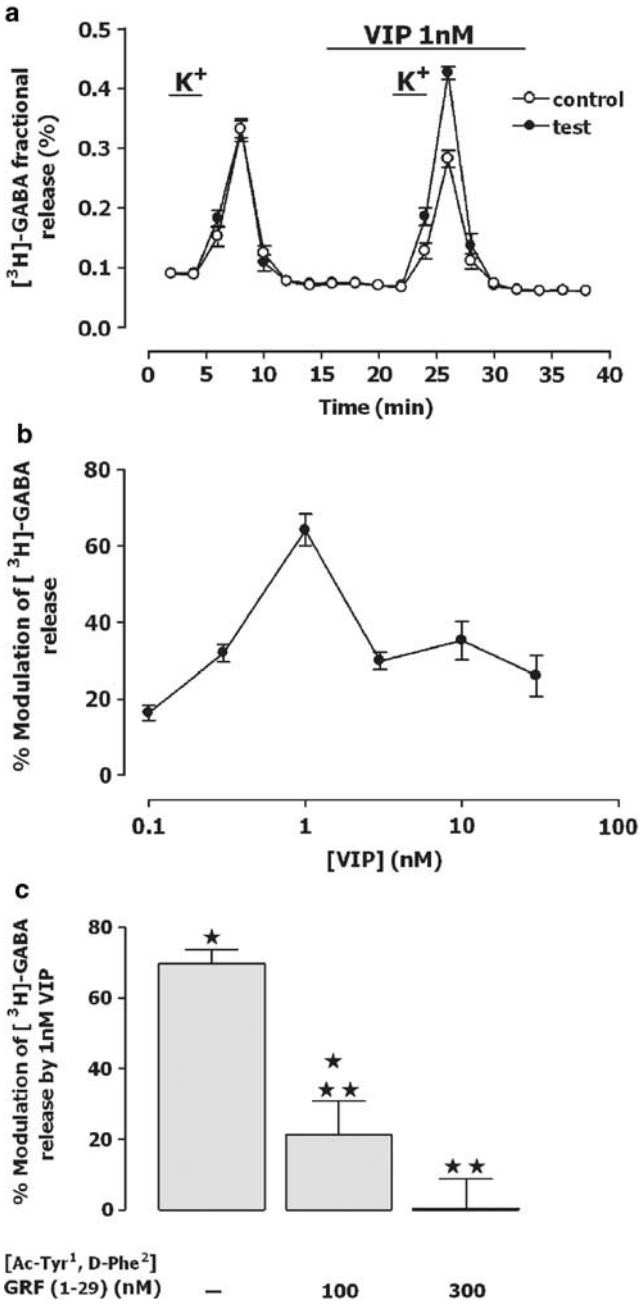

Figure 1 VIP enhances K^+-evoked [^{3}H]GABA release from isolated hippocampal nerve terminals. (a) Time course of averaged [^{3}H]GABA release experiments in which the effect of VIP (1 nM) was tested. Nerve terminals were labelled with [^{3}H]GABA as described in Methods. Release was evoked by two 2-min pulses of 28 mM KCl as indicated by the horizontal bars. VIP was added to the test chambers before S_2, as indicated by the horizontal bar, whereas it was not added to the parallel control chambers. Each point represents the mean ± s.e.m. of the results obtained in 16 experiments performed in duplicate. The S_2/S_1 ratio was 0.837 ± 0.015 in control and 1.249 ± 0.036 in test conditions. (b) Concentration–response curve for the enhancement of K^+-evoked [^{3}H]GABA release caused by VIP in hippocampal nerve terminals. The effect of VIP was calculated by comparing the S_2/S_1 ratio obtained in test (presence of VIP during S_2) and in control conditions. Each point represents the mean ± s.e.m. of results obtained in 5–8 experiments, except for 1 nM VIP ($n = 16$). The effect of VIP is statistically significant ($P < 0.01$, Student's *t*-test, as compared with 0%) for all concentrations tested. (c) Ability of the nonselective VIP receptor antagonist [Ac-Tyr^1, D-Phe^2] GRF(1–29) to inhibit the action of VIP (1 nM) on [^{3}H]GABA release. Each bar represents the mean ± s.e.m. of results obtained in 4–16 experiments. *$P < 0.01$ (Student's *t*-test) as compared with 0%. **$P < 0.01$ (ANOVA, followed by Dunnett's multiple comparison test) as compared with the effect of 1 nM VIP in the absence of other drugs (left column).

tion to 200 nM during S_2 decreased the S_2/S_1 ratio from 0.837 ± 0.051 to 0.435 ± 0.012, inhibiting the evoked tritium release by $51.3 \pm 5.0\%$ ($n=6$, $P<0.01$). Thus, under these experimental conditions, [^{3}H]GABA release was nearly 50% Ca^{2+}-dependent, as previously observed by Kirk & Richardson (1994). Synaptosome disruption was small since only $13.7 \pm 0.5\%$ ($n=2$) of the total lactate dehydrogenase activity (EC 1.1.1.27) was detected at the end of experiments upon superfusion of the synaptosomes in closed circuit.

When VIP (1 nM) was present during S_2 (Figure 1a, circles in full), the S_2/S_1 ratio (1.249 ± 0.036, $n=16$) was significantly greater ($63.8 \pm 4.0\%$, $n=16$, $P<0.01$) compared with the S_2/S_1 ratio obtained in parallel control chambers (0.837 ± 0.015, $n=16$, Figure 1a, open circles). As shown in Figure 1b, VIP facilitation of K^+-evoked [^{3}H]GABA release increased with the peptide concentration from 0.3 to 1 nM. At concentrations higher than 1 nM, the facilitation was smaller reaching a plateau that was maintained from 3 to 30 nM concentrations. No significant modification on the basal tritium release by VIP was observed for any of the concentrations tested.

To evaluate if VIP enhancement of [^{3}H]GABA release was occurring through activation of VIP receptors, we tested the influence of the VIP receptor antagonist [Ac-Tyr^1, D-Phe^2] GRF(1–29) upon VIP action. VIP was used at the concentration (1 nM) that caused maximum excitatory effect on [^{3}H]GABA release. In the presence of [Ac-Tyr^1, D-Phe^2] GRF(1–29) (100 nM), a concentration previously described to block the action of VIP (Liu *et al*., 2000), the effect of VIP was strongly ($67.2 \pm 11.2\%$) inhibited (Figure 1c), but still led to an increase of $21.3 \pm 9.2\%$ ($n=4$, $P<0.01$) on K^+-evoked [^{3}H]GABA release. In the presence of a higher concentration of antagonist (300 nM), the effect of VIP on K^+-evoked [^{3}H]GABA release was abolished ($0.4 \pm 8.3\%$, $n=4$, $P>0.01$) (Figure 1c).

To elucidate if VIP enhancement of [^{3}H]GABA release was occuring at calcium dependent component of release, we tested VIP action upon removal of extracellular calcium (200 nM Ca^{2+} and 0.5 mM EGTA). Under these conditions, the effect of VIP on [^{3}H]GABA release was significantly ($P<0.05$) reduced to $23.6 \pm 5.5\%$ ($n=7$), suggesting that VIP effect is mostly dependent on extracellular calcium.

VIP did not change K^+-evoked [^{3}H]glutamate release from isolated nerve terminals

In synaptosomes incubated with [^{3}H]glutamate, the amount of tritium retained at the beginning of sample collection was $(4.28 \pm 0.32) \times 10^7$ Bq $mg^{-1}_{protein}$ ($n=32$). The basal release of tritium was $(4.03 \pm 0.54) \times 10^5$ Bq $mg^{-1}_{protein}$ ($n=12$), giving a fractional release of 1.001 ± 0.0323 ($n=12$) for the first sample collected. Stimulation of synaptosomes with 20 mM K^+ for 30 s caused a nearly two-fold increase in the amount of tritium released (Figure 2a) and the S_2/S_1 ratio in control conditions was 0.832 ± 0.034 ($n=12$). The K^+-evoked [^{3}H]glutamate release was mostly Ca^{2+}-dependent since the reduction of the extracellular calcium concentration to 200 nM together with the addition of 0.5 mM EGTA to the superfusion solution during S_2 decreased the S_2/S_1 ratio by $71.4 \pm 5.3\%$ ($n=4$, $P<0.01$).

When VIP (0.1–100 nM) was present during S_2 (Figure 2), the S_2/S_1 ratios were not significantly different ($P>0.05$) from the S_2/S_1 ratio obtained in parallel control chambers, indicating that VIP caused no appreciable change in K^+-evoked [^{3}H]glutamate release. Also, no significant modifications on the basal tritium release upon addition of VIP were observed for any of the concentrations tested.

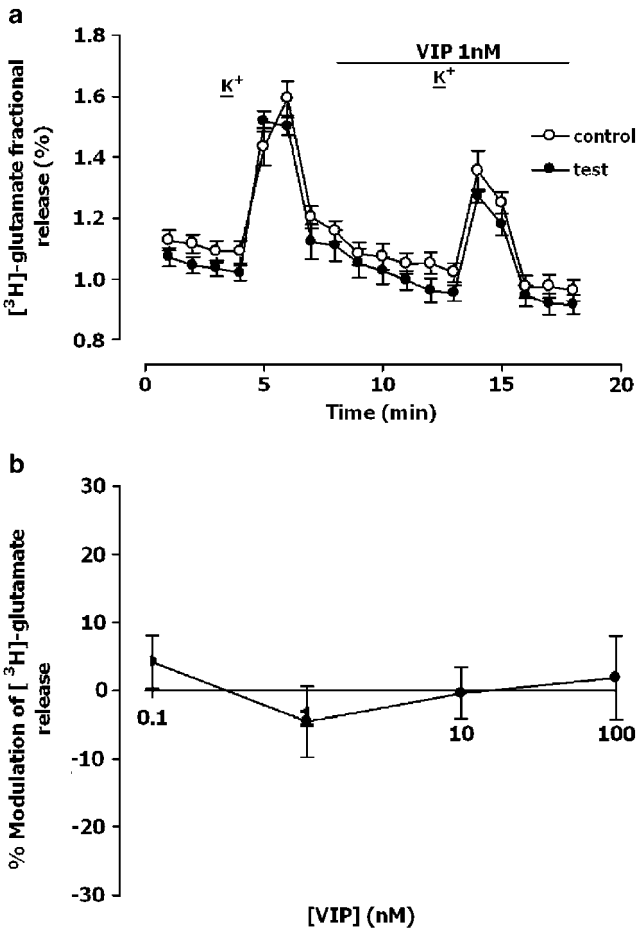

Figure 2 VIP does not change K^+-evoked [^{3}H]glutamate release from hippocampal nerve terminals. (a) Time course of averaged [^{3}H]glutamate release experiments in which the effect of VIP (1 nM) was tested. Nerve terminals were labelled with [^{3}H]glutamate as described in Methods. Release was evoked by two 30-s pulses of 20 mM KCl as indicated by the horizontal bars. VIP was added to the test chambers before S_2, as indicated by the horizontal bar, whereas it was not added to the parallel control chambers. Each point represents the mean ± s.e.m. of the results obtained in five experiments performed in duplicate. The S_2/S_1 ratio was 0.752 ± 0.045 in control and 0.730 ± 0.042 in test conditions. (b) Effect of different VIP concentrations, calculated by comparing the S_2/S_1 ratio obtained in test (presence of VIP during S_2) and in control conditions. Each point represents the mean ± s.e.m. of results obtained in 4–5 experiments.

VIP facilitates synaptic transmission to CA1 pyramidal cells

When applied to hippocampal slices, VIP (1 nM) caused a consistent and reversible increase in the slope ($23.7 \pm 1.1\%$, $n=16$, $P<0.05$, Figure 3b) of the field epsps and in the amplitude ($20.3 \pm 1.7\%$, $n=6$, $P<0.05$, Figure 3f) of orthodromically evoked PSs recorded in the CA1 area. The maximal effect of the neuropeptide was attained 20–25 min after starting VIP perfusion and was reversed within 25–40 min after starting VIP washout (Figure 3). For higher VIP concentrations, the

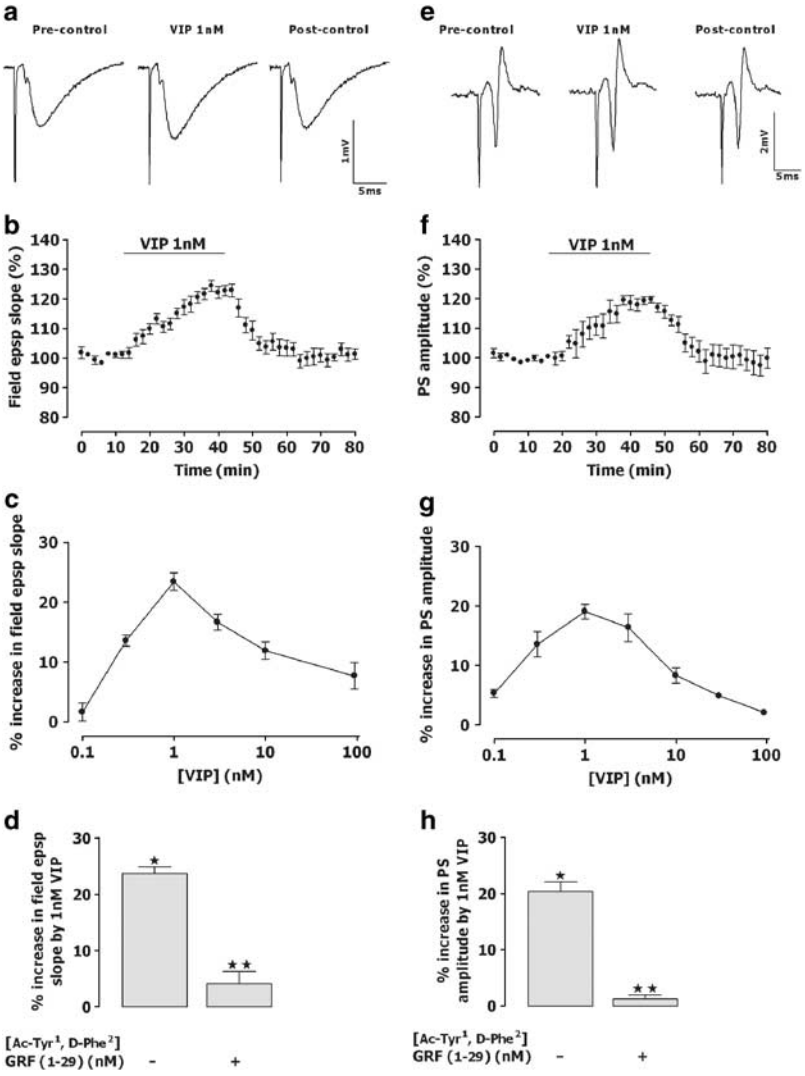

Figure 3 VIP increases synaptic transmission in rat hippocampal slices. (a) Recordings of field epsps. Each trace is composed of the stimulus artefact followed by the presynaptic volley and the epsp and is the average of eight consecutive responses obtained in one typical experiment (from left to right): before perfusion of VIP, at the maximum effect of VIP (1 nM) and after 40 min washout of VIP. Field epsps were recorded in the *stratum radiatum* as described in Methods. (b) Averaged time course of changes in field epsp slope caused by VIP (1 nM), which was added to the slices as indicated by the horizontal bar. Each point represents the mean ± s.e.m. of the results obtained in 16 individual experiments. 100% represents the averaged field epsp slopes recorded for the last 8 min before VIP application. (c) Concentration–response curve for the enhancement of field epsp slope caused by VIP. Each point represents the mean ± s.e.m. of results obtained in 4–16 experiments. The effects were statistically significant ($P<0.05$, Student's *t*-test, as compared with 0%) for all concentrations tested, except for the lowest (0.1 nM). (d) Ability of the nonselective VIP receptor antagonist [Ac-Tyr^1, D-Phe^2] GRF(1–29) (300 nM) to inhibit the modulatory action of VIP (1 nM) on field epsps. (e) Recordings of orthodromically evoked PSs. Each trace is the average of eight consecutive responses obtained in one typical experiment (from left to right): before perfusion of VIP, at the maximum effect of VIP (1 nM) and after 40 min washout of VIP. PSs were recorded in the *stratum piramidale* as described in Methods. (f) Averaged time course of changes in PS amplitude caused by VIP (1 nM), which was added to the slices as indicated by the horizontal bar. Each point represents the mean ± s.e.m. of the results obtained in six individual experiments. 100% represents the averaged PS amplitudes recorded for the last 8 min before VIP application. (g) Concentration–response curve for the enhancement of PS amplitude caused by VIP. Each point represents the mean ± s.e.m. of results obtained in 3–6 experiments. The effect of VIP was statistically significant ($P<0.05$, Student's *t*-test, as compared with 0%) for all concentrations tested, except for the lowest (0.1 nM) and the highest (100 nM). (h) Ability of the nonselective VIP receptor antagonist [Ac-Tyr^1, D-Phe^2] GRF(1–29) to inhibit the modulatory action of VIP (1 nM) on PSs. $*P<0.01$ (Student's *t*-test) as compared with 0%. $**P<0.01$ (ANOVA, followed by Dunnett's multiple comparison test) as compared with the effect of VIP (1 nM) in the absence of other drugs (left column).

maximal effect was reached earlier (15–20 min after starting VIP perfusion) and was followed by a subsequent small decrease in the response, which occurred still before the washout period.

The concentration–response curves for VIP (0.1–100 nM) action on synaptic transmission and pyramidal cell action potentials are shown in Figure 3c and g, respectively. The increase in the slope of the field epsps caused by VIP was biphasic (Figure 3c), increasing with the concentration from 0.1 to 1 nM VIP and then decreasing when tested in concentrations up to 100 nM. The concentration–response curve for the effects of VIP on PS amplitude (Figure 3g) was similar.

The effects of VIP (1 nM) on field epsp slope and PS amplitude were abolished in the presence of a supramaximal concentration (300 nM) of the VIP receptor antagonist [Ac-Tyr[1], D-Phe[2]] GRF(1–29) (Figure 3d and h).

Role of GABAergic transmission in the effects of VIP on synaptic transmission to CA1 pyramidal cells

In order to evaluate if disinhibition, caused by increased GABAergic transmission to interneurones, could be the cause for the effect of VIP on epsps and PSs, we tested if suppression of GABAergic transmission (using both $GABA_A$ and $GABA_B$ receptor blockade) could influence the excitatory actions of VIP. $GABA_A$ and $GABA_B$ receptors were antagonized using supramaximal concentrations of the selective antagonists picrotoxin (50 μM) and CGP55845 (1 μM). When applied together to slices, these drugs increased field epsp slope by $32.2 \pm 9.4\%$ ($n=5$) and PS amplitude by $35.6 \pm 3.1\%$ ($n=4$). Before adding VIP in the presence of the GABA antagonists, stimulus intensities were adjusted in order to obtain responses of nearly the same magnitude (50% of maximum) as that before the GABA receptor antagonists and the preparation was allowed to stabilize with the new stimulus intensity. VIP was tested in the concentration (1 nM) that caused maximal enhancement of field epsp slope and PS amplitude. As illustrated in Figure 4a (right panel) and c, the effect of VIP (1 nM) on the field epsp slope was abolished (% change of the field epsp slope: $3.2 \pm 1.7\%$, $n=5$, $P>0.05$) when GABAergic transmission had been prevented; under similar conditions, the effect VIP (1 nM) on PS amplitude was strongly reduced (% change of the PS amplitude: $11.9 \pm 2.9\%$, $n=4$, $P<0.05$, Figure 4b (right panel) and d). Previous studies described that $GABA_B$ receptor activation potentiates the effects of VIP on cAMP production in the cerebral cortex (Watling & Bristow, 1986). To exclude the possibility that a reduction in GABAergic tonus at $GABA_B$ receptors could mediate a decrease in VIP efficacy, we tested VIP action upon suppression of $GABA_A$ (but not $GABA_B$) transmission. When applied to slices, picrotoxin (50 μM) increased field epsp slope by $44.8 \pm 12.9\%$ ($n=5$) and PS amplitude by $72.8 \pm 21.0\%$ ($n=5$); stimulus intensities were then adjusted (see above). The effect of VIP (1 nM) on the field epsp slope was abolished (% change of the field epsp slope: $2.0 \pm 0.8\%$, $n=5$, $P>0.05$, Figure 4a (middle panel) and c) when $GABA_A$ receptor-mediated transmission had been prevented and the effect VIP (1 nM) on PS amplitude was strongly reduced (% change of the PS amplitude: $9.0 \pm 0.9\%$, $n=5$, $P<0.05$, Figure 4b (middle panel) and d).

VIP did not affect paired-pulse facilitation but facilitated paired-pulse inhibition in hippocampal area CA1

When two consecutive stimulation pulses separated by a 50 ms interval were delivered to the Schaffer collateral/commissural pathway in the CA1 area, the slope of the field epsp evoked by the second (test, S_2) stimulation pulse was 1.6–2.0 times larger than the field epsp slope evoked by the first (conditioning, S_1) stimulation pulse (Figure 5a, left panel). This is known as PPF and is thought to arise due to the persistence of residual calcium (caused by the first stimulation pulse) in the excitatory presynaptic terminals, which causes an enhanced release of neurotransmitter in response to the second stimulation pulse

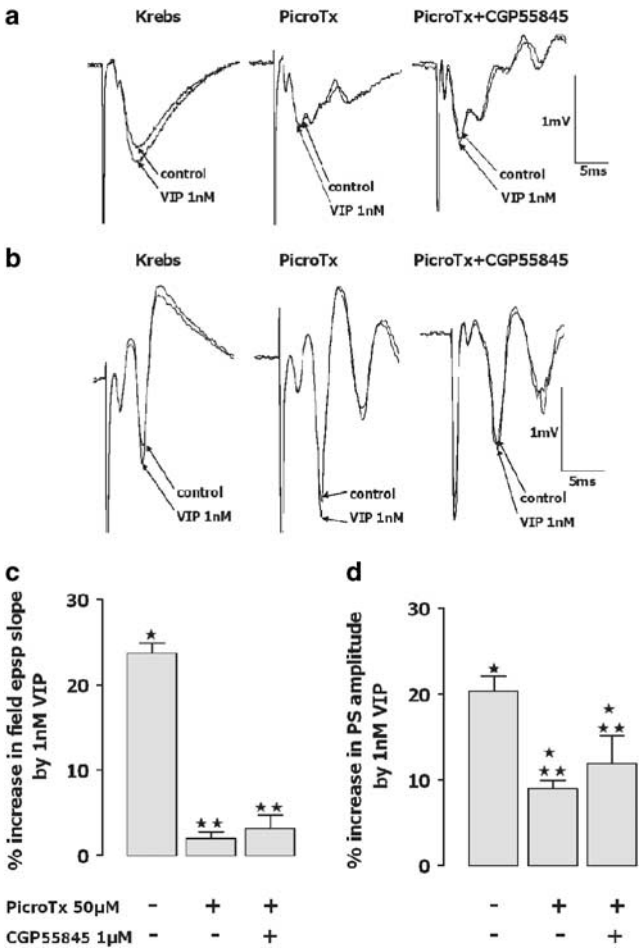

Figure 4 Blockade of GABAergic transmission suppressed the excitatory action of VIP (1 nM) on synaptic transmission in the CA1 area of the rat hippocampus. Recordings of field epsps (a) and orthodromically evoked PSs (b) to evaluate the action of VIP either applied alone (left) in the presence of the $GABA_A$ receptor antagonist picrotoxin (50 μM) (PicroTx, middle) or in the presence of both the $GABA_A$ and $GABA_B$ receptor antagonists picrotoxin (50 μM) and CGP55845 (1 μM) (PicroTx + CGP55845, right). In each panel, responses immediately before perfusion of VIP (control) and after 30 min superfusion with VIP (1 nM) are superimposed. Each trace is the average of eight consecutive responses obtained in one typical experiment. Blockade of GABAergic transmission caused an increase in excitability reflected by a characteristic change in the shape of field epsps and PSs (a and b, middle and right panels). Averaged effects of VIP on field epsp slope (c) and PS amplitude (d), in the absence, in the presence of the $GABA_A$ receptor antagonist and in the presence of both the $GABA_A$ and $GABA_B$ receptor antagonists. The $GABA_A$ receptor antagonist picrotoxin (PicroTx, 50 μM) alone or together with the $GABA_B$ receptor antagonist CGP55845 (1 μM) was present for at least 30 min before VIP application. VIP effects were calculated as described in Methods. Each bar represents the mean ± s.e.m. of results obtained in 5–16 experiments for epsps and 4–6 experiments for PSs. $*P<0.01$ (Student's t-test) as compared with 0%. $**P<0.01$ (ANOVA, followed by Dunnett's multiple comparison test) as compared with the effect of 1 nM VIP in the absence of other drugs (left column).

(Katz & Milledi, 1968; Zucker, 1989) at synapses where the release probability is low, such as the CA3–CA1 synapses under the stimulation conditions used (Creager *et al*., 1980; Dunwiddie & Haas, 1985; Debanne *et al*., 1996). PPF in the CA1 area of the hippocampus has also been attributed to paired-pulse depression in the fast inhibitory postsynaptic

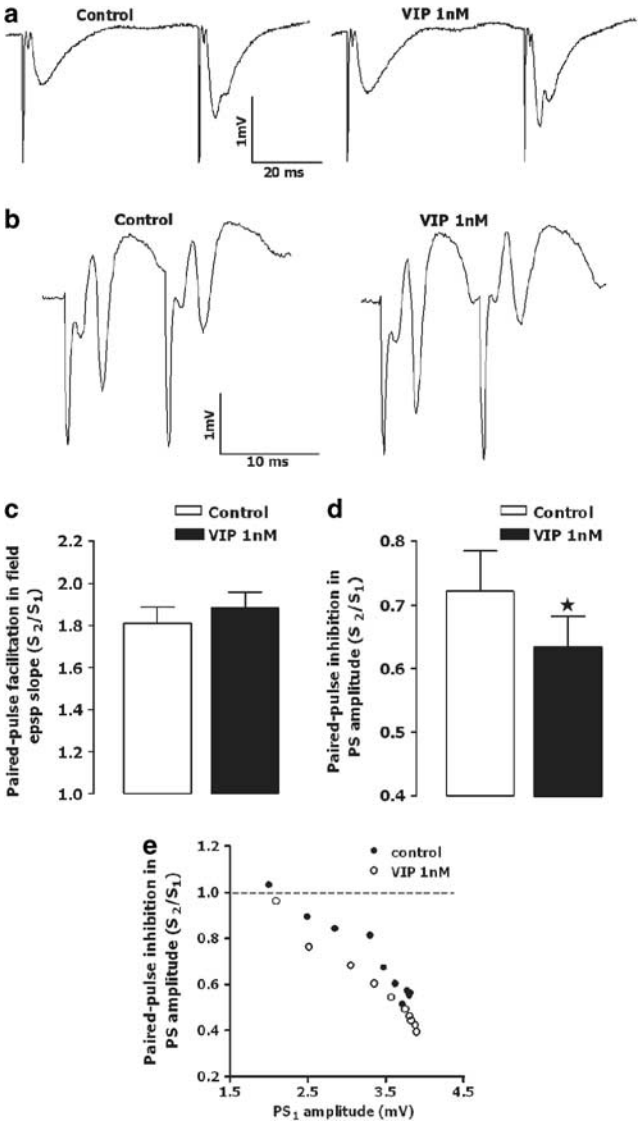

Figure 5 VIP increases PPI but does not affect PPF in hippocampal CA1 area. PPF was induced by applying two consecutive stimuli with 50 ms interval once every 15 s while recording field epsps; PPI was induced by applying two consecutive stimuli with 10 ms interval once every 15 s while recording PSs. Representative recordings of field epsp pairs (a) obtained when using the PPF paradigm and of PS pairs (b) obtained when using the PPI paradigm are shown. Averages of eight consecutive responses obtained prior to VIP superfusion (control) and during superfusion of VIP (1 nM) are depicted. (c) VIP (1 nM) did not affect field epsp slope ratio (S_2/S_1) between responses evoked by the first (conditioning) and the second (test) stimulation pulses when using the PPF paradigm. (d) Facilitation by 1 nM VIP of averaged PS amplitude ratio (S_2/S_1) between responses evoked by the first (conditioning) and the second (test) stimulation pulses when using the PPI paradigm. In (c) and (d), each bar represents the mean ± s.e.m. of results obtained in four and six experiments, respectively. $^*P<0.01$ (paired Student's *t*-test) as compared with S_2/S_1 ratio in control conditions. (e) Individual experiment where different stimulation intensities were used to evoke PPI in the absence and in the presence of 1 nM VIP. S_2/S_1 ratios (ordinates) are plotted against the amplitude of the first (conditioning, PS_1) PS (abscissas). Note that in the presence of VIP, the curve is shifted to the left, evidencing an enhancement of PPI for similar amplitudes of the conditioning spike.

potential (IPSP) (Davies *et al*., 1990; Nathan & Lambert, 1991). This is thought to be due to $GABA_B$ autoreceptor-mediated depression of neurotransmitter release at interneurone–pyramidal cell synapses (Davies *et al*., 1990; Lambert & Wilson, 1994). Therefore, facilitation of glutamatergic transmission together with depression of GABAergic transmission might be contributing to extracellularly recorded PPF of field epsp slope in the CA1 area of the hippocampus. Since most synapses in the *stratum radiatum* are excitatory (Megiás *et al*., 2001), changes in PPF of field epsp slope are most likely predominantly due to presynaptic changes in glutamate release. When applied to hippocampal slices, VIP (1 nM) did not significantly change PPF ($n=4$, $P>0.05$, Figure 5c), but rather increased the slope of the field epsp evoked by the conditioning and the test stimuli to the same extent (see Figure 5a), suggesting that changes in glutamate release probability are not involved in VIP-mediated enhancement of synaptic transmission in the CA1 area of the hippocampus. This is in agreement with the observation that VIP does not change glutamate release from hippocampal nerve terminals (see above).

When two consecutive stimulation pulses separated by a 10 ms interval were delivered to the Schaffer collateral/commissural pathway in the CA1 area and the responses were recorded at the *stratum piramidale*, we could observe that the amplitude of the PS response evoked by the second (test, S_2) stimulation pulse was about 2/3 of the amplitude of the response to the first (conditioning, S_1) stimulation pulse (Figure 5b, left panel). This phenomenon is usually known as PPI and is most consistently observed at interpulse intervals less than 40 ms (Creager *et al*., 1980; Dunwiddie & Haas, 1985) and when responses are recorded at the *stratum piramidale*. This might result from recurrent inhibition of CA1 pyramidal cells, which is active 4–10 ms after pyramidal cell spike (Miles *et al*., 1996), due to activation of inhibitory synapses that are mostly located at proximal dendrites and at the soma (Megiás *et al*., 2001). An increase in feedforward inhibition to pyramidal cells due to repetitive stimulation of Schaffer collaterals has also been described (Buzsaki & Eidelberg, 1982), Therefore, PPI is attributed to increased activation of local feedforward and feedback inhibitory interneurones, releasing GABA as a neurotransmitter (Higgins & Stone, 1993) and changes in PPI are usually interpreted as a consequence of changes in GABAergic transmission. When VIP (1 nM) was applied to hippocampal slices, PPI was significantly ($n=6$, $P<0.01$) enhanced (Figure 5d).

PPI can depend on the size of the first PS, since the greater the number of cells spiking, the greater the feedback inhibition. Therefore, the increase in PS amplitude caused by VIP could lead to an increase in feedback inhibition not attributable to a direct action of VIP in inhibitory circuits. To access this possibility, we used different stimulation intensities to evoke PPI and plotted the paired-pulse ratio against the size of the initial PS (PS_1). Results obtained in one experiment are shown in Figure 5e and it can be seen that VIP caused a left horizontal shift in the curve. This means that for equivalent PS_1 amplitudes, PPI is still enhanced by VIP. A similar result was observed in seven out of eight experiments. Altogether, these experiments show that VIP enhances PPI in the hippocampus, further supporting the involvement of modulation of GABAergic transmission in the action of VIP.

Modulation by VIP of GABAergic currents in interneurones and pyramidal cells in hippocampal area CA1

Since the action of VIP on synaptic transmission was dependent on GABAergic transmission, we investigated if VIP was involved in postsynaptic modulation of GABAergic input to interneurones and to pyramidal cells. When applied for 2 s to the cells, at a holding potential of −70 mV, the $GABA_A$ receptor agonist muscimol (30 μM) caused an inward current. Repeated application of muscimol caused a rundown of these currents in interneurones. This rundown was minimized using a 3 min interval between applications and with this procedure the rundown, taken from the comparison between the first and the ninth currents, was never greater than 15%. Because of this small rundown of the currents, the effect of VIP was quantified taking as control the average of the current amplitudes before and after VIP application (see Methods). Muscimol-evoked currents in pyramidal cells (Figure 6a) ranged from 165 to 705 pA. VIP (1 nM) caused an increase of 15.9±2.9% ($P<0.05$, Figure 6c) in the amplitude of the currents in five out of nine cells tested, having no appreciable effect on the remaining four cells. In interneurones, muscimol-evoked currents (Figure 6b) ranged from 95 to 320 pA. VIP (1 nM) caused an increase of 36.4±8.7% ($P<0.05$, Figure 6d) in the amplitude of these currents in eight out of 10 cells tested. An inhibition of the currents occurred in the two other cells tested (−14.1±0.6%, $P<0.05$). These two cells were located more distally than the eight cells in which VIP caused a marked increase in the amplitude of GABAergic currents. Therefore, the predominant action of VIP on *stratum radiatum* interneurones seems to be a facilitation of GABAergic currents, which is more intense ($P<0.05$) than the one observed in pyramidal cells.

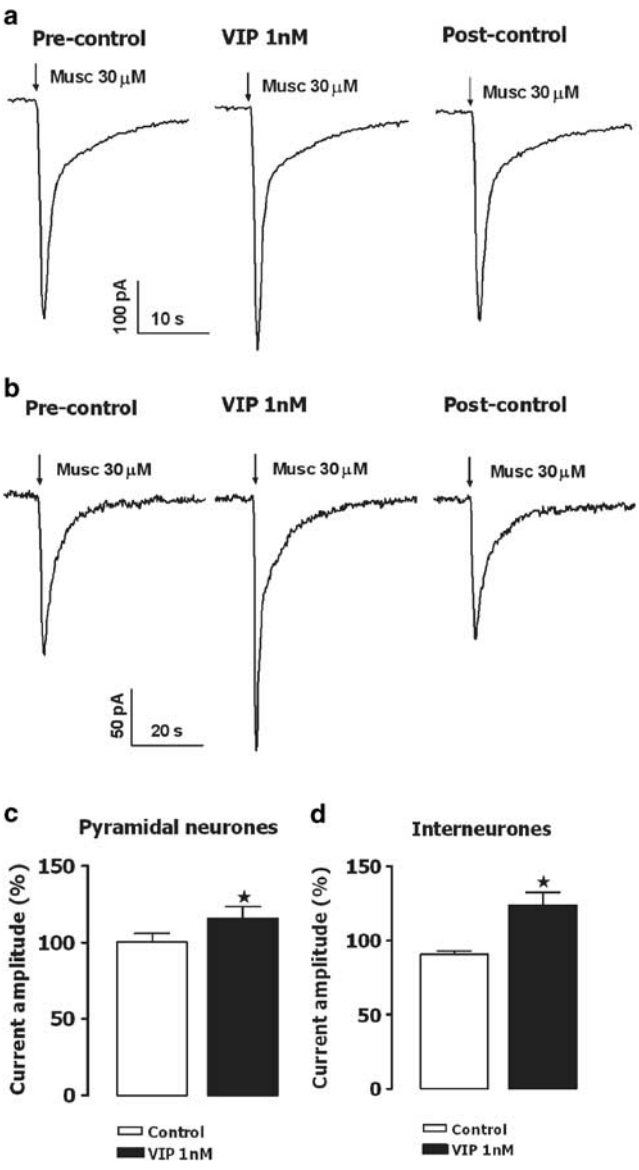

Figure 6 VIP increases muscimol-evoked GABAergic currents in both interneurones and pyramidal neurones in the CA1 area of the hippocampus. Enhancement caused by VIP (1 nM) on muscimol-evoked GABAergic currents recorded from a pyramidal cell (a) and from an interneurone (b). Muscimol (30 μM) was pressure-applied for 2 s every 3 min. Each trace represents individual responses obtained in one typical experiment (from left to right): before perfusion of VIP, after 8 min superfusion with VIP and after 8 min washout of VIP. In these cells, VIP facilitation of GABAergic currents was 16.3% (a) and 70.5% (b). Averaged modifications caused by 1 nM VIP on the amplitude of GABAergic currents in pyramidal cells (c) and interneurones (d) in the CA1 area are shown. 100% corresponds to the amplitude of the current recorded immediately before VIP application. The third current during VIP application (1 nM) was compared with the average (control) between the current immediately before VIP application and the third current after VIP washout (third and ninth currents; see Methods). Each bar represents the mean±s.e.m. of results obtained in five experiments (c, pyramidal cells) or eight experiments (d, interneurones). *$P<0.01$ (Student's *t*-test) as compared with the control.

Discussion

The main findings in the present work are that VIP at low nanomolar concentrations (1) facilitates evoked GABA release from hippocampal synaptosomes, leaving evoked glutamate release unaffected; (2) increases synaptic transmission to pyramidal cell dendrites and pyramidal cell firing in the CA1 area of the hippocampus in a way that is dependent on GABAergic transmission; (3) facilitates PPI, leaving PPF unaffected; (4) facilitates muscimol-evoked $GABA_A$ currents in interneurones. Altogether, these observations strongly suggest that VIP-mediated enhancement of GABAergic transmission to interneurones (occurring both at pre- and postsynaptic sites of action) is the main mechanism leading to enhancement of synaptic transmission at Schaffer collateral – CA1 pyramidal cell synapses.

The finding that VIP (0.3–30 nM) increases K^+-evoked [^{3}H]GABA release from hippocampal synaptosomes provides evidence for a presynaptic action of VIP at GABAergic nerve terminals in the hippocampus. Previous electrophysiological observations show that iontophoretic application of VIP enhances the frequency of miniature inhibitory postsynaptic currents in pyramidal hippocampal neurones in culture (Wang *et al*., 1997). However, these authors used pressure ejection as a means to deliver VIP to the cells, which does not allow a proper assessment of the VIP concentrations active at GABAergic nerve terminals. In this study, we used bath application of VIP and all VIP-mediated actions here described could be observed at low nanomolar concentrations of VIP (0.3–30 nM), known to activate VIP-selective receptors

(Inagaki *et al*., 1994; Couvineau *et al*., 1996), which suggests a physiological role of this peptide in the hippocampus. Indeed, the maximum effect of VIP was observed at 1 nM, higher concentrations causing a smaller effect. This could be due to receptor desensitization (Staun-Olsen *et al*., 1982; Marie *et al*., 2003) or to an interplay between $VPAC_1$ and $VPAC_2$ receptors (Cunha-Reis *et al*., 2002a). Interestingly, Brenneman & Eiden (1986) described a protective effect of VIP against TTX-induced neuronal cell death in dissociated spinal cord cultures with a biphasic response, reaching a maximum at low VIP concentration (0.1 nM), and decreasing for higher VIP concentrations.

The absence of a VIP-mediated effect on K^+-evoked [^{3}H]glutamate release from hippocampal synaptosomes was not surprising since VIP is expressed only in interneurones, which do not synapse with nerve terminals of pyramidal cells but around their cell bodies; in the case of the areas rich in glutamatergic nerve terminals such as the *stratum radiatum* in CA1 and CA3 areas, VIP-immunoreactive interneurones synapse exclusively with other interneurones (Acsády *et al*., 1996b). Thus, our observations provide functional data consistent with the previous morphological data and further exclude the possibility of the presence of axoaxonic synapses (see Acsády *et al*., 1996a), where presynaptic modulation of glutamatergic transmission by VIP could occur. The observation that the neuropeptide could not change PPF of synaptic transmission in the CA1 area of the hippocampus is also consistent with absence of presynaptic modulation of glutamatergic transmission by VIP in the hippocampal CA1 area.

We observed that VIP (0.3–100 nM) increases synaptic transmission, assessed either by changes in field epsp slope or PS amplitude, in the CA1 area of the hippocampus. The effects are quantitatively smaller than those obtained for VIP action on [^{3}H]GABA release. This could be due to differences in the methodologies used (K^+ stimulation vs electrical stimulation) or differences in accessibility of a large molecule such as VIP to the preparation (synaptosomes vs slices). The observed enhancement of synaptic transmission by VIP is consistent with the excitatory actions of VIP originally described by Haas & Gähwiler (1992). In the present work, the VIP concentration (1 nM) required for maximal effect is 100 times lower than the concentrations earlier described to have an effect on synaptic transmission or pyramidal cell excitability (Haas & Gähwiler, 1992; Yanovski *et al*., 1997), yet these studies did not provide concentration–response curves for VIP action. Interestingly, VIP effects for these low nanomolar concentrations are consistent with the IC_{50} for VIP receptors, and are in a range that can be considered to activate selectively VIP receptors vs other receptors of the family such as the PAC_1 receptor for PACAP and secretin receptors (see Harmar *et al*., 1998 for review).

We show that the facilitation caused by VIP on synaptic transmission (field epsps recording) is completely dependent on $GABA_A$ receptor-mediated transmission, suggesting that it could be due to disinhibition, caused by an increase in the inhibitory input to interneurones. This disinhibition most likely occurs at synapses between VIP-immunoreactive interneurones and interneurones in the *stratum radiatum*, since local VIP application to the targets of VIP-immunoreactive interneurones projecting to the *Oriens/Alveus* border in CA1 has been described to decrease transmission to pyramidal cell dendrites (Yanovski *et al*., 1997). In contrast with what occurred with the action of VIP on field epsps, the action of VIP on PSs was attenuated but not fully prevented by blockade of GABAergic transmission, which suggests that the action of VIP on pyramidal cells may arise from two mechanisms, one involving disinhibition at Schaffer collateral – pyramidal cell synapses (present work), and another involving the direct action on pyramidal cells described by Haas & Gähwiler (1992). The observation that VIP (1 nM) causes an increase in PPI of pyramidal cell excitability further suggests that facilitation of GABAergic transmission is involved in VIP-mediated excitatory actions in the hippocampus. Furthermore, we observed that VIP increases $GABA_A$ inhibitory currents in *stratum radiatum* interneurones. This provides direct evidence for a postsynaptic action of VIP leading to inhibition of interneurones, and further supports the hypothesis that enhancement of synaptic transmission to pyramidal cells arises from GABA-mediated suppression of the inhibitory input to pyramidal neurones. Since it is known that the targets of VIP-immunoreactive interneurones that project to the *stratum radiatum* are inhibitory neurones, which in turn target pyramidal cell dendrites in the *stratum radiatum* (Acsády *et al*., 1996b), an enhancement of the inhibitory input to these interneurones would lead to disinhibition at pyramidal cell dendrites causing an enhancement in extracellularly recorded transmission (field epsps and PSs). Our data showing that VIP increases muscimol-evoked currents in interneurones together with those showing that VIP enhances GABA release from nerve terminals imply that both pre- and postsynaptic mechanisms contribute to the VIP-mediated increase of the inhibitory input to interneurones.

VIP also caused a small, although not consistent, facilitation of GABAergic currents in pyramidal cells. This is not fully in agreement with the observations of Wang *et al*. (1997), who found no postsynaptic action of VIP on GABAergic transmission to pyramidal cells. However, in the culture model, some of the VIP-mediated actions might be lost since VIP-immunoreactive neurones seem to be present in a very well-defined position in inhibitory circuits in the hippocampus (Acsády *et al*., 1996a, b; Papp *et al*., 1999), which are likely disrupted or changed in cell culture. Perisomatic inhibition of pyramidal cells has a relevant role in suppressing repetitive discharge of sodium-dependent action potentials (Miles *et al*., 1996) and therefore in the control of firing frequency. Perisomatic inhibitory actions of VIP might counterbalance the dendritic and postsynaptic depolarizing actions of VIP on pyramidal cells. Such balance of opposing mechanisms has been proposed to play a key role in neural information coding (Freund & Buzsaki, 1996; Buzsáki, 1997).

Interestingly, VIP-immunoreactive neurones that project to the *stratum radiatum* are targets for median raphe serotonergic fibres (Papp *et al*., 1999). Serotonin (5-HT) has been shown to decrease inhibition to pyramidal cell dendrites (Segal, 1990; Schmitz *et al*., 1995), and therefore shares the actions now described for VIP in the hippocampus. VIP/CCK-immunoreactive basket cells innervating the perisomatic region of pyramidal cells are also targets for serotonergic fibres projecting from the median raphe nucleus (Papp *et al*., 1999), express ionotropic $5\text{-}HT_3$ receptors (Morales & Bloom, 1997) and are depolarized by $5\text{-}HT_3$ receptor agonists (McMahon & Kauer, 1997), which are also able to increase perisomatic GABAergic transmission to pyramidal cells (Ropert & Guy, 1991). Again, our observations that VIP can

directly facilitate GABAergic input to pyramidal cell neurones are in agreement with perisomatic inhibitory actions of serotonin 5-HT_3 agonists in pyramidal cells (Ropert, 1988; Ropert & Guy, 1991). It thus appears that VIP could be an important intermediate/mediator of hippocampal actions of serotonin, which leads to increased excitability and plasticity at pyramidal cell dendrites but reduces pyramidal cell firing capability. VIP-immunoreactive interneurones are also innervated by GABAergic septal afferents (Papp *et al*., 1999) that promote perisomatic disinhibition of hippocampal pyramidal cells through inhibition of basket cell activity (Tóth *et al*., 1997). The fact that VIP-immunoreactive interneurones and in particular VIP/CCK-immunoreactive basket cells are avoided by cholinergic (excitatory) septal afferents (Papp *et al*., 1999), which innervate profusely other interneurone populations (Gulyás *et al*., 1999), suggests that selective inhibition of VIP-immunoreactive interneurones could mediate suppression of serotonergic raphe afferent activity in situations requiring predominance of septal influence over raphe influence of hippocampal circuits (see King *et al*., 1998; Kitchigina *et al*., 1999; Vinogradova *et al*., 1999).

In conclusion, VIP decreases inhibitory transmission to pyramidal cell dendrites in the hippocampus, leading to facilitation of synaptic transmission through disinhibition. Also, VIP increases perisomatic inhibition of pyramidal cells, and this might suppress repetitive firing of pyramidal cells. These effects are due to concerted pre- and postsynaptic actions of VIP on different interneurone populations. Enhanced disinhibition caused by VIP probably increases plasticity at pyramidal cell dendrites with consequences for selective hippocampal functions as learning and memory. The ability of VIP to regulate the activity of interneurones might also be relevant for the modulation of specific hippocampal activity patterns, such as theta, beta and gamma oscillations, and to the regulation of physiological states such as arousal that are associated with the actions of the serotonergic raphe system.

We acknowledge the Institute of Physiology, FML, for animal housing facilities and Dr R.A. Cunha for introducing Diana Cunha-Reis to the GABA release technique. Diana Cunha-Reis was in receipt of an FCT PhD fellowship. This work was supported by FCT and Deutsche Forschungsgemeinschaft (IL-20/9-2).

References

ACSÁDY, L., ARABADZISZ, D. & FREUND, T.F. (1996a). Correlated morphological and neurochemical features identify different subsets of vasoactive intestinal polypeptide-immunoreactive interneurons in the rat hippocampus. *Neuroscience,* **73,** 299–315.

ACSÁDY, L., GÖRCS, T.J. & FREUND, T.F. (1996b). Different populations of vasoactive intestinal polypeptide-immunoreactive interneurons are specialised to control pyramidal cells or interneurons in the hippocampus. *Neuroscience,* **73,** 317–334.

ANDERSON, W.W. & COLLINGRIDGE, G.L. (1997). A data acquisition program for on-line analysis of long-term potentiation and long-term depression. *Soc. Neurosci. Abstr.,* **23,** 665.

BARRY, P.H. (1994). JPCalc, a software package for calculating liquid junction potential corrections in patch-clamp, intracellular, epithelial and bilayer measurements and for correcting junction potential measurements. *J. Neurosci. Methods,* **51,** 107–116.

BRENNEMAN, D.E. & EIDEN, L.E. (1986). Vasoactive intestinal peptide and electrical activity influence neuronal survival. *Proc. Natl. Acad. Sci. U.S.A.,* **83,** 1159–1162.

BUZSÁKI, G. (1997). Functions for interneuronal nets in the hippocampus. *Can. J. Physiol. Pharmacol.,* **75,** 508–515.

BUZSAKI, G. & EIDELBERG, E. (1982). Direct afferent excitation and long-term potentiation of hippocampal interneurons. *J. Neurophysiol.,* **48,** 597–607.

COUVINEAU, A., ROUYERD-FESSARD, C., MAORET, J.J., GAUDIN, P., NICOLE, P. & LABURTHE, M. (1996). Vasoactive intestinal peptide (VIP_1) receptor: three non-adjacent amino acids are responsible for species selectivity with respect to recognition of peptide histidine isoleucineamide. *J. Biol. Chem.,* **271,** 12795–12800.

CREAGER, R., DUNWIDDIE, T. & LYNCH, G. (1980). Paired-pulse and frequency facilitation in the CA1 region of the *in vitro* rat hippocampus. *J. Physiol. (Lond.),* **299,** 409–424.

CUNHA, R.A., CONSTANTINO, M.D. & RIBEIRO, J.A. (1997). Inhibition of [^{3}H]-γ-aminobutyric acid release by kainate receptor activation in rat hippocampal synaptosomes. *Eur. J. Pharmacol.,* **323,** 167–172.

CUNHA-REIS, D., SEBASTIAO, A.M. & RIBEIRO, J.A. (2002a). Activation of both VPAC1 and VPAC2 receptors is involved in VIP modulation of K+-evoked [^{3}H]-GABA release from hippocampal synaptosomes. Proceedings of the 33rd Meeting of the Portuguese Society of Pharmacology, C46.

CUNHA-REIS, D., SEBASTIAO, A.M. & RIBEIRO, J.A. (2002b). Modulation of synaptic transmission by VIP in the CA1 area of the hippocampus is dependent on GABAergic transmission and on both PKA and PKC activities. *Br. J. Pharmacol.,* **135,** 83P (Abstr.).

DAVIES, C.H., DAVIES, S.N. & COLLINGRIDGE, G.L. (1990). Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. *J. Physiol. (Lond.),* **424,** 513–531.

DEBANNE, D., GUÉRINEAU, N.C., GÄHWILER, B.H. & THOMPSON, S.M. (1996). Paired-pulse facilitation and depression at unitary synapses in rat hippocampus: quantal fluctuation affects subsequent release. *J. Physiol. (Lond.),* **491,** 163–176.

DUNWIDDIE, T.V. & HAAS, H.L. (1985). Adenosine increases synaptic facilitation in the *in vitro* rat hippocampus: evidence for a presynaptic site of action. *J. Physiol. (Lond.),* **369,** 365–377.

EDWARDS, F.A., KONNERTH, A., SAKMANN, B. & TAKAHASHI, T. (1989). A thin slice preparation for patch-clamp recordings from neurones of the mammalian central nervous system. *Pflugers Arch.,* **414,** 600–612.

FREUND, T.F. & ANTAL, M. (1988). GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. *Nature,* **336,** 170–173.

FREUND, T.F. & BUZSAKI, G. (1996). Interneurons of the hippocampus. *Hippocampus,* **6,** 345–470.

GULYÁS, A.I., ACSÁDY, L. & FREUND, T.F. (1999). Structural basis of the cholinergic and serotonergic modulation of GABAergic neurons in the hippocampus. *Neurochem. Int.,* **34,** 359–372.

HAAS, H.L. & GÄHWILER, B.H. (1992). Vasoactive intestinal polypeptide modulates neuronal excitability in hippocampal slices of the rat. *Neuroscience,* **47,** 273–277.

HARMAR, A.J., ARIMURA, A., GOZES, I., JOURNOT, L., LABURTHE, M., PISEGNA, J.R., RAWLINGS, S.R., ROBBERECHT, P., SAID, S.I., SREEDHARAN, S.P., WANK, S.A. & WASHEK, J.A. (1998). International Union of Pharmacology. XVIII. Nomenclature of receptors for the vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. *Pharmacol. Rev.,* **50,** 265–270.

HIGGINS, M.J. & STONE, T.W. (1993). Bicuculline-resistant paired-pulse inhibition in the rat hippocampal slice. *Br. J. Pharmacol.,* **109,** 1164–1168.

INAGAKI, N., YOSHIDA, H., MIZUTA, M., MIZUNO, N., FUJII, Y., GONOI, T., MIYAZAKI, J.-I. & SEINO, S. (1994). Cloning and functional characterization of third pituitary adenylate cyclase-activating polypeptide receptor subtype expressed in insulin-secreting cells. *Proc. Natl. Acad. Sci. U.S.A.,* **91,** 2679–2683.

KATZ, B. & MILLEDI, R. (1968). The role of calcium in neuromuscular facilitation. *J. Physiol. (Lond.),* **195,** 481–492.

KEIDING, R., HORDER, M., GERHART, W., PITKANEN, E., TEHHUNEN, R., STÖMME, J.H., THEODERESON, L., WALDENSTRÖM, J., TRYDING, N. & WESTLUND, L. (1974). Recommended methods for the determination of four enzymes in the blood. *Scan. J. Clin. Lab. Invest.,* **33,** 291–300.

KING, C., RECCE, M. & O'KEEFE, J. (1998). The rhythmicity of cells of the medial septum/diagonal band of Broca in the awake freely moving rat: relationships with behaviour and hippocampal theta. *Eur. J. Neurosci.,* **10,** 464–477.

KIRK, I.P. & RICHARDSON, P.J. (1994). Adenosine A_{2A} receptor-mediated modulation of striatal [^{3}H]-GABA and [^{3}H]-acethylcholine release. *J. Neurochem.,* **62,** 960–966.

KITCHIGINA, V.F., KUDINA, T.A., KUTYREVA, E.V. & VINOGRADOVA, O.S. (1999). Neuronal activity of the septal pacemaker of theta rhythm under the influence of stimulation and blockade of the median raphe nucleus in the awake rabbit. *Neuroscience,* **94,** 453–463.

LAMBERT, N.A. & WILSON, W.A. (1994). Temporally distinct mechanisms of use-dependent depression at inhibitory synapses in the rat hippocampus *in vitro*. *J. Neurophysiol.,* **72,** 121–130.

LIU, D.-L., CUEVAS, J. & ADAMS, D.J. (2000). VIP and PACAP potentiation of nicotinic ACh-evoked currents in rat parasympathetic neurons is mediated by G-protein activation. *Eur. J. Neurosci.,* **12,** 2243–2251.

LONART, G., JANZ, R., JOHNSON, K.M. & SÜDHOF, T.C. (1996). Mechanisms of action of rab3A in mossy fiber LTP. *Neuron,* **21,** 1141–1150.

MARIE, J.C., ROUYER-FESSARD, C., COUVINEAU, A., NICOLE, P., DEVAUD, H., EL BENNA, J. & LABURTHE, M. (2003). Serine 447 in the carboxyl tail of human VPAC1 receptor is crucial for agonist-induced desensitization but not internalization of the receptor. *Mol. Pharmacol.,* **64,** 1565–1574.

MCMAHON, L.L. & KAUER, J.A. (1997). Hippocampal interneurons are excited *via* serotonin-gated ion channels. *J. Neurophys.,* **78,** 2493–2502.

MEGIÁS, M., EMRI, Z.S., FREUND, T.F. & GULYÁS, A.I. (2001). Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience,* **102,** 527–540.

MILES, R., TÓTH, K., GULYÁS, A.I., HÁJOS, N. & FREUND, T.F. (1996). Differences between somatic and dendritic inhibition in the hippocampus. *Neuron,* **16,** 815–823.

MORALES, M. & BLOOM, F.E. (1997). The 5-HT3 receptor is present in different subpopulations of GABAergic neurons in the rat telencephalon. *J. Neurosci.,* **17,** 3157–3167.

NATHAN, T. & LAMBERT, J.D.C. (1991). Depression of the fast IPSP underlies paired-pulse facilitation in area CA1 of the rat hippocampus. *J. Neurophysiol.,* **66,** 1704–1715.

PAPP, E.C., HÁJOS, N., ACSÁDY, L. & FREUND, T.F. (1999). Medial septal and median raphe innervation of vasoactive intestinal polypeptide-containing interneurons in the hippocampus. *Neuroscience,* **90,** 369–382.

PETERSON, G.L. (1977). A simplification of the protein assay method of Lowry *et al*. which is more generally applicable. *Anal. Biochem.,* **83,** 346–356.

ROPERT, N. (1988). Inhibitory action of serotonin in CA1 hippocampal neurons *in vitro*. *Neuroscience,* **26,** 69–81.

ROPERT, N. & GUY, N. (1991). Serotonin facilitates GABAergic transmission in the CA1 area of rat hippocampus *in vitro*. *J. Physiol. (Lond.),* **441,** 121–136.

SCHMITZ, D., EMPSON, R.M. & HEINEMANN, U. (1995). Serotonin reduces inhibition *via* 5-HT1A receptors in area CA1 of rat hippocampal slices *in vitro*. *J. Neurosci.,* **15,** 7217–7225.

SEBASTIÃO, A.M., DE MENDONCA, A., MOREIRA, T. & RIBEIRO, J.A. (2001). Activation of synaptic NMDA receptors by action potential-dependent release of transmitter during hypoxia impairs recovery of synaptic transmission on reoxygenation. *J. Neurosci.,* **21,** 8564–8571.

SEGAL, M. (1990). Serotonin attenuates a slow inhibitory postsynaptic potential in rat hippocampal neurons. *Neuroscience,* **36,** 631–641.

STAUN-OLSEN, P., OTTENSEN, B., BARTELS, P.D., NIELSEN, M.H., GAMMELTOFT, S. & FAHRENKRUG, J. (1982). Receptors for vasoactive intestinal polypeptide on isolated synaptosomes from rat cerebral cortex. Heterogeneity of binding and desensitization of receptors. *J. Neurochem.,* **39,** 1242–1251.

TÓTH, K., FREUND, T.F. & MILES, R.I. (1997). Disinhibition of rat hippocampal pyramidal cells by GABAergic afferents from the septum. *J. Physiol. (Lond.),* **500,** 463–474.

VERTONGEN, P., SCHIFFMANN, S.N., GOURLET, P. & ROBBERECHT, P. (1997). Autoradiographic visualization of the receptor subclasses for vasoactive intestinal polypeptide (VIP) in rat brain. *Peptides,* **18,** 1547–1554.

VINOGRADOVA, O.S., KITCHIGINA, V.F., KUDINA, T.A. & ZENCHENKO, K.I. (1999). Spontaneous activity and sensory responses of hippocampal neurons during persistent theta-rhythm evoked by median raphe nucleus blockade in rabbit. *Neuroscience,* **94,** 745–753.

WANG, H.-L., LI, A. & WU, T. (1997). Vasoactive intestinal polypeptide enhances the GABAergic synaptic transmission in cultured hippocampal neurons. *Brain Res.,* **746,** 294–300.

WATLING, K.J. & BRISTOW, D.R. (1986). GABAB receptor-mediated enhancement of vasoactive intestinal peptide-stimulated cycic AMP production in slices of the rat cerebral cortex. *J. Neurochem.,* **46,** 1755–1762.

WIRKNER, K., ASSMANN, H., KOLES, L., GEREVICH, Z., FRANKE, H., NORENBERG, W., BOEHM, R. & ILLES, P. (2000). Inhibition by adenosine A_{2A} receptors of NMDA but not AMPA currents in rat neostriatal neurons. *Br. J. Pharmacol.,* **130,** 259–269.

YANOVSKI, Y., SERGEEVA, O.A., FREUND, T.F. & HAAS, H.L. (1997). Activation of interneurons at the stratum oriens/alveus border suppresses excitatory transmission to apical dendrites in the CA1 area of the mouse hippocampus. *Neuroscience,* **77,** 87–96.

ZUCKER, R.S. (1989). Short-term synaptic plasticity. *Annu. Rev. Neurosci.,* **12,** 13–31.

(Received June 7, 2004
Revised August 3, 2004
Accepted August 26, 2004)