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MODULATION BY VASOACTIVE INTESTINAL PEPTIDE (VIP) OF SEROTONIN$_1$ RECEPTORS IN MEMBRANES FROM RAT HIPPOCAMPUS[1]

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Abstract

The vasoactive intestinal peptide (VIP) has been located in various structures of the rat brain, but few actions of the peptide have been reported as yet. Because VIP might interact with classical neurotransmitter systems in the central nervous system as it does in the periphery, we investigated whether VIP can modulate serotonin (5-HT$_1$) receptors in membrane preparations obtained from brain areas which contain various amounts of VIP and 5-HT receptors. The presence of bacitracin alone, which protects VIP from proteolytic degradation, decreases the affinity of [^{3}H]5-HT binding in almost all of the structures tested. Scatchard analysis indicates that, in the presence of bacitracin, VIP significantly decreases the affinity and increases the number of specific high affinity binding sites for [^{3}H]5-HT in the dorsal hippocampus. VIP induces a dose-dependent increase in the number of 5-HT$_1$ receptors with a maximal response of 60% with 10^{-7} M VIP. At the same concentration, neither secretin nor glucagon modifies 5-HT$_1$ receptor density. No effect of VIP is observed in the ventral hippocampus, parietal cortex, whole hypothalamus, and midbrain. This effect of VIP is not observed when bacitracin is omitted, and the presence of calcium ions does not alter the efficacy of the VIP effect. No effect of VIP is obtained on [^{3}H]spiperone binding assayed with 10 μM mianserin to define specific binding. The present data suggest that some of the effects of 5-HT in the hippocampus may be modulated by VIP.

The vasoactive intestinal peptide (VIP) has been detected recently in various structures of the central nervous system (CNS) by means of immunohistochemical, electron microscopic, and radioimmunological techniques (Larsson et al., 1976b; Fuxe et al., 1977; Besson et al., 1979; Loren et al., 1979; Rostene et al., 1982). High concentrations of VIP are found in the cerebral cortex, the hippocampus, and the hypothalamus, whereas the mesencephlon contains low amounts of the peptide. The presence of VIP in neurons and its release from nerve terminals by various depolarizing agents (Giachetti et al., 1976; Emson et al., 1978; Besson et al., 1979, 1982), as well as the presence of specific binding sites (Taylor and Pert, 1979; Staun-Olsen et al., 1982) coupled to adenylate cyclase (Deschodt-Lanckman et al., 1977; Quik et al., 1978; Borghi et al., 1979), attributes a possible neurotransmitter function to VIP in the CNS. However, specific functions of VIP in the CNS are not well documented. VIP has been shown to induce excitation of cortical neurons (Phillis et al., 1978), glycogenolysis in mouse cortical slices (Magistretti et al., 1981), and vasodilatation of cerebral blood vessels (Larsson et al., 1976a).

Serotonin (5-HT)-positive nerve terminals originating from the midbrain raphe nuclei have been shown to innervate various forebrain regions such as the cortex, the hippocampus, and the hypothalamus (Steinbusch, 1981). Moreover, those structures, as well as the midbrain, have been shown to contain high to moderate amounts of 5-HT receptors (Peroutka and Snyder, 1981; Biegon et al., 1982). Therefore, the present study was designed to test whether VIP can modulate the binding of 5-HT to membranes from various brain areas.

Materials and Methods

Male Sprague-Dawley rats (220 to 240 gm, Charles River Breeding Laboratories, Wilmington, MA) were

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used. The animals were killed between 9:00 and 11:00 AM (light/dark cycle: 5.00 to 19.00, lights on) by decapitation, and various brain regions such as dorsal and ventral hippocampus, parietal cortex, whole hypothalamus, and pontine regions of the midbrain were rapidly dissected on ice and kept frozen at −70°C. Membrane preparation and assay procedure are based on the method of Bennett and Snyder (1976) as modified by Nelson et al. (1978) and Biegon and McEwen (1982). In brief, frozen tissues were thawed and homogenized in 50 mM Tris-HCl buffer, pH 7.6, in a glass-Teflon homogenizer (Arthur H. Thomas Co., Philadelphia, PA). The homogenate was centrifuged at 30,000 × *g* for 15 min, washed with buffer, homogenized with a Brinkmann Polytron (setting 7, for 10 sec), and centrifuged again. After two washes, the membrane pellet was resuspended in 50 mM Tris-HCl buffer and incubated for 10 min at 37°C to facilitate destruction of endogenous 5-HT monoamine oxidase. The membranes were then centrifuged for 10 min at 30,000 × *g*, and the supernatant was discarded and replaced by an equal volume of 50 mM Tris-HCl buffer, pH 7.4, containing 0.2 mg/ml of ascorbate and 10^{-4} M pargyline (Saber Laboratories, Morton Grove, IL). The membrane preparation was homogenized with a Polytron (5 sec) and preincubated for 45 min at 25°C under agitation in the presence or absence of bacitracin (2×10^{-5} M; Sigma Chemical Co., St. Louis, MO) plus or minus various concentrations of VIP.

Incubation was performed for 10 min at 37°C in the presence of increasing concentrations of [^{3}H]5-HT (New England Nuclear, Boston, MA; 27 to 30 Ci/mmol; 0.6 to 6 nM; 0.25 ml), 0.5 ml of tissue homogenate (100 to 500 μg of protein/sample), and 0.25 ml of buffer or 20 μM (5 μM final) unlabeled 5-HT (Sigma) to evaluate nonspecific binding. The incubation reaction was terminated by transferring the tubes to an ice water bath. Tissues were then filtered through Whatman GF/B glass fiber filters using a Brandel Cell Harvester (Brandel Instruments, Gaithersburg, MD). Filtration was followed by three 2-sec washes with ice-cold buffer, 2 sec apart (which is equivalent to 10 ml). Filters were placed in scintillation vials containing 5 ml of Liquiscint (National Diagnostics, Inc., Somerville, NJ), incubated for 10 min at 50°C, and then cooled and counted in a β-scintillation counter at 32% efficiency.

[^{3}H]Spiperone (New England Nuclear; 30 Ci/mmol) binding was also carried out under similar experimental conditions except that the preincubation and incubation (in the presence of increasing concentrations of [^{3}H] spiroperidol, 0.09 nM to 0.76 nM) were performed at 25°C in 50 mM Tris-HCl buffer, pH 7.25, containing NaCl (120 mM), KCl (5 mM), and 0.1% ascorbate, for 45 min and 30 min, respectively. Nonspecific binding was obtained by addition of 10^{-5} M mianserin (Organon Diagnostics, West Orange, NJ).

Protein content was determined by the method of Bradford (1976). Statistical analyses were carried out using the unpaired Student's *t* test.

Results

5-HT$_1$ binding assay. The characteristics of 5-HT$_1$ binding (by means of [^{3}H]5-HT), in terms of B_{max} and K_D, are similar to those already reported (Bennett and Snyder, 1976; Nelson et al., 1978; Biegon and McEwen, 1982), and the pharmacological specificity of the assay was previously validated in our laboratory (Biegon and McEwen, 1982).

Effect of bacitracin on 5-HT binding. The presence of bacitracin (2×10^{-5} M), a proteolytic inhibitor (Desbuquois and Cuatrecasas, 1972; McKelvy et al., 1976) in the pre- and incubation medium induces a significant increase in the K_D for [^{3}H]5-HT, e.g., a decrease in the affinity for the ligand and no change in the number of binding sites. The same tendency is observed in the dorsal hippocampus, but it is not significant (Table I).

Effect of VIP on 5-HT binding in various brain structures. Binding of [^{3}H]5-HT to membranes from the ventral hippocampus (control K_D, 4.9 ± 0.8 nM; 616 ± 92 fmol/mg of protein versus VIP K_D, 5.9 ± 0.4; B_{max}, 702 ± 97 (6)), the parietal cortex (5.7 ± 0.6; 426 ± 31 versus 6.7 ± 0.3; 508 ± 55(3)), the whole hypothalamus (6.4 ± 0.6; 692 ± 38 versus 7.2 ± 1.5; 630 ± 37(3)), and the pontine formation of the midbrain (5.8 ± 0.1; 521 ± 56 versus 7.1 ± 0.5; 505 ± 80(3)) is not affected by 10^{-7} or 2×10^{-7} M VIP. In contrast, as shown in Figure 1, incubation of VIP (10^{-7} M) in the presence of bacitracin with membranes from the dorsal hippocampus significantly ($p < 0.01$) decreases the affinity for [^{3}H]5-HT (control, 3.8 ± 0.4 nM versus VIP, 6.0 ± 0.4 (4)) and increases the number of binding sites (460 ± 20 fmol/mg

TABLE I

Effect of bacitracin on [^{3}H]5-HT binding parameters in various brain structures

Membranes from various brain areas were preincubated and incubated with increasing concentrations of [^{3}H]5-HT in Tris buffer with or without 2×10^{-5} M bacitracin. This concentration of bacitracin is able to reduce VIP degradation by at least 50% in media containing brain slices without producing the detergent effects noted at higher bacitracin concentrations (Besson et al., 1982). Bacitracin at 2×10^{-5} M is routinely used to study effects of VIP on prolactin release from pituitary (e.g., Rotsztejn et al., 1981). Nonspecific binding was estimated with 5 μM unlabeled 5-HT.

	Dorsal Hippocampus		Ventral Hippocampus		Cerebral Cortex		Whole Hypothalamus		Pontine Midbrain	
	k_D[a]	B_{max}[b]	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}
Control	3.2 ± 0.2	574 ± 41	2.7 ± 0.2	567 ± 51	3.0 ± 0.7	430 ± 54	3.5 ± 0.8	605 ± 59	4.2 ± 0.1	492 ± 22
Bacitracin (2×10^{-5} M)	3.8 ± 0.4	462 ± 22	4.9 ± 0.8[c]	616 ± 92	5.7 ± 0.6[c]	426 ± 31	6.4 ± 0.6[c]	692 ± 38	5.8 ± 0.1[c]	521 ± 56
	(4)[d]		(6)		(3)		(3)		(3)	

[a] Dissociation constant in nanomolar concentrations.

[b] Number of binding sites in fmol/mg of protein.

[c] $p < 0.05$ versus control using the unpaired Student's *t* test.

[d] Numbers in parentheses, number of experiments.

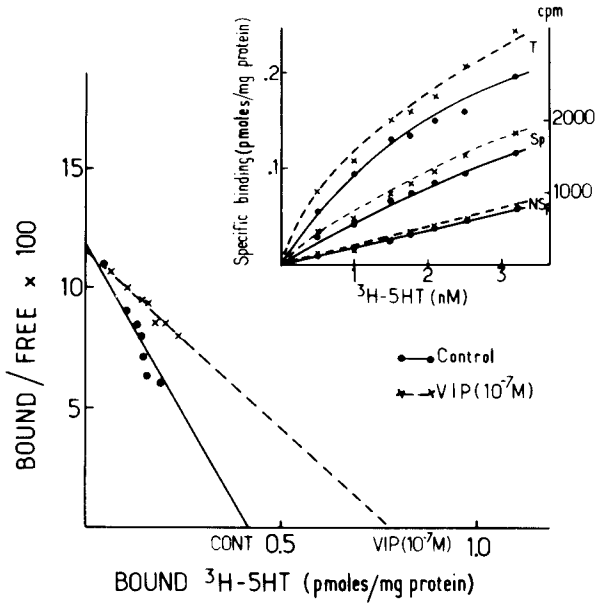

Figure 1. Effect of VIP on [^{3}H]5-HT binding in the dorsal hippocampus. Saturation curves (*T*, total; *Nsp*, nonspecific binding with 5 μM unlabeled 5-HT; *Sp*, specific binding) and Scatchard representation of the data are presented in the absence (control, ●—●) and in the presence of 10^{-7} M VIP (x---x) in the preincubation (45 min, 25°C) and incubation (10 min, 37°C with increasing concentrations of [^{3}H]5-HT) medium.

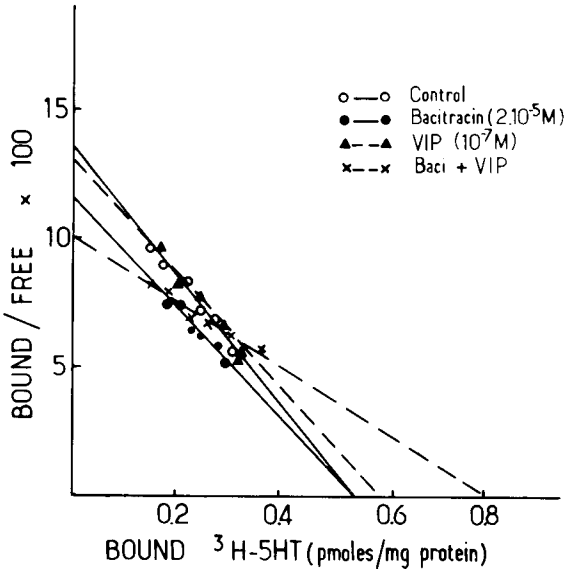

Figure 2. Effect of VIP on [^{3}H]5-HT-specific binding in the dorsal hippocampus in the presence or absence of bacitracin. VIP (10^{-7} M) was incubated in the absence (▲---▲) or presence (x---x) of 2×10^{-5} M bacitracin. These groups are compared to Tris buffer (control, O—O) or Tris buffer containing 2×10^{-5} M bacitracin (●—●).

of protein versus 750 ± 10 (4)). This effect of VIP can be observed only if bacitracin is present in the incubation medium (Fig. 2).

Dose response of VIP on 5-HT biding in the dorsal hippocampus. VIP, at concentrations ranging from 3×10^{-9} M to 10^{-7}M, produces a dose-dependent increase in [^{3}H]5-HT-specific binding in the dorsal hippocampus with a maximum increase of approximately 60% at 10^{-7}M VIP. The effect declines at higher concentrations of the peptide (Fig. 3).

Specificity of the effect of VIP in the dorsal hippocampus. Other peptides, such as secretin and glucagon, which share structural analogies with VIP (Said and Mutt, 1972), do not modify the number of 5-HT-binding sites at an identical concentration of VIP (10^{-7}M) which produces the maximum effect. However, secretin at that concentration appears to decrease the affinity of [^{3}H]5-HT binding in the dorsal hippocampus (Fig. 4).

Effect of calcium on VIP-induced increase of 5-HT binding. Because calcium ions have been reported recently to increase the number of 5-HT-binding sites (Mallat and Hamon, 1982), we tested whether the effect of VIP can be modified by the presence of calcium ions in the incubation medium. As shown in Figure 5, the presence of 4 mM $CaCl_2$ dramatically increases the number of binding sites for [^{3}H]5-HT in the dorsal hippocampus concomitant with a slight increase in the affinity. However, the efficacy of VIP to increase 5-HT_1-specific binding is not affected by the presence of calcium.

Effect of VIP on [^{3}H]spiperone binding in the dorsal hippocampus. In contrast to the effect of VIP on [^{3}H]5-HT binding, the binding of [^{3}H]spiperone in the dorsal hippocampus is not modified by identical amounts of VIP (data not shown).

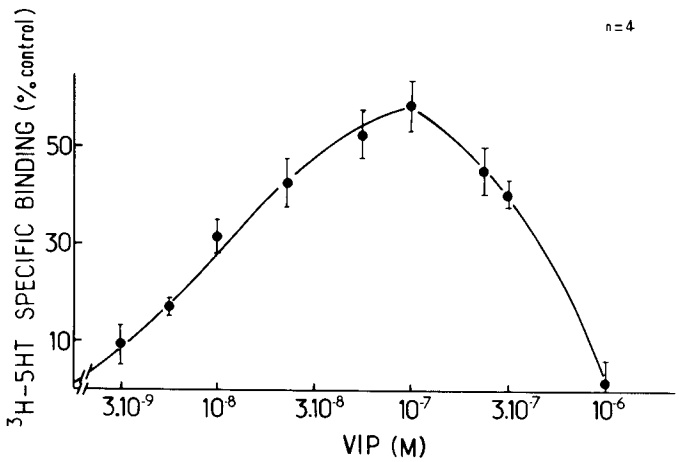

Figure 3. Dose-dependent stimulation of [^{3}H]5-HT binding in the dorsal hippocampus with increasing concentrations of VIP. [^{3}H]5-HT was used at 4.2 nM final concentration in the presence of 2×10^{-5} M bacitracin. Results are expressed as percentage of increase versus control and represent the mean ± SEM of four individual values for each VIP concentration.

Discussion

The present data are suggestive of a possible role of VIP as a modulator of 5-HT-binding sites in the dorsal hippocampus. This effect is remarkably specific. An anatomical specificity is obtained, since the effect of VIP is only observed in the dorsal hippocampus but not in the ventral hippocampus, nor in other brain regions which contain high amounts of VIP (cerebral cortex) or low amounts (midbrain). By means of quantitative autoradiography of [^{3}H]5-HT on brain slices, we have demonstrated that the increase of [^{3}H]5-HT-binding sites induced by VIP is located in a discrete region of dorsal hippocampus, the dorsal subiculum (Rostene et al., 1983).

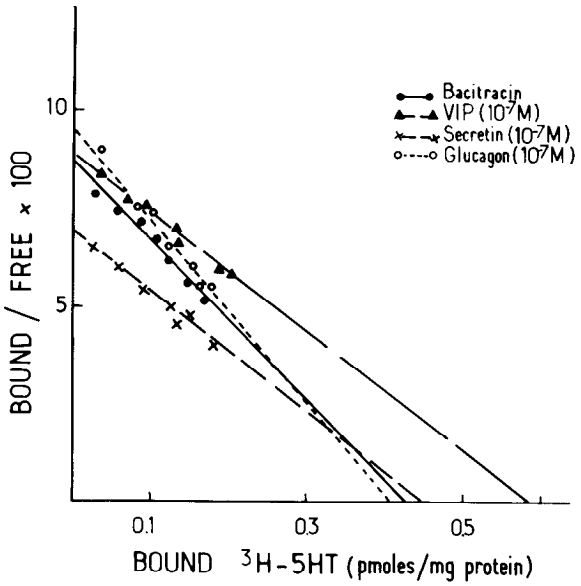

Figure 4. Scatchard representation of peptide specificity on [^{3}H]5-HT binding in the dorsal hippocampus. Secretin and glucagon (10^{-7} M) were added under experimental conditions similar to those for VIP (10^{-7}M) in the presence of 2×10^{-5} M bacitracin.

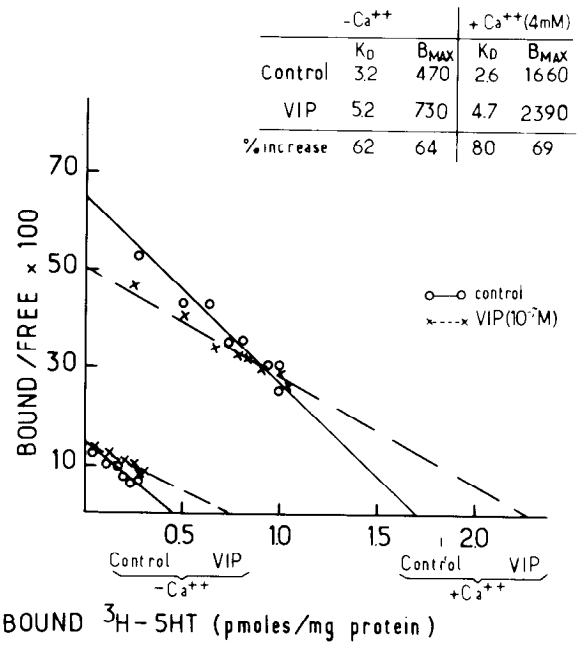

Figure 5. Effect of calcium ions on [^{3}H]5-HT binding in the dorsal hippocampus in the presence or absence of 10^{-7} M VIP. Preincubation and incubation were carried out with or without 4 mM $CaCl_2$ into the Tris buffer in the presence of 2×10^{-5} M bacitracin.

The effect of VIP seems to be mediated by specific VIP receptors, since they have been found in high concentrations in the hippocampus (Taylor and Pert, 1979), and other peptides such as secretin and glucagon, structurally related to VIP (Said and Mutt, 1972), do not produce any significant change in the number of 5-HT_1-binding sites at concentrations similar to those for VIP. However, it is interesting to note that, like VIP, secretin appears to decrease the affinity for 5HT_1 biding. Since secretin has been shown to inhibit the binding of [^{125}I] VIP but with affinities 1/100 to 1/1000 those of unlabeled VIP (Robberecht et al., 1978; Prieto et al., 1979; Taylor and Pert, 1979), further studies may be able to show that much higher concentrations of secretin may mimic the effect of VIP on [^{3}H]5-HT binding. Whether that has any physiological significance remains to be determined.

The dose response concentration of VIP to increase specific binding for 5-HT in the dorsal hippocampus is in good agreement with effective doses of VIP, which have been shown to produce a physiological effect; for instance, on both cyclic AMP accumulation and prolactin release from pituitary cells (Rotsztejn et al., 1980, 1981), a maximum effect of VIP is observed with 10^{-7} M VIP. A biphasic effect of VIP obtained with increasing concentrations of VIP has already been observed on cyclic AMP productin in a human prolactin-secreting pituitary tumor (Bataille et al., 1979).

In the dorsal hippocampus, VIP seems to affect [^{3}H] 5-HT binding, whereas it does not modify [^{3}H]spiperone binding. By using nanomolar concentrations of [^{3}H]5-HT, it has been postulated that high affinity 5-HT receptors (or 5-HT_1 receptors) are labeled as opposed to the 5-HT_2 receptors which have low (μM) affinity for 5-HT and are preferentially labeled by spiperone (Peroutka and Snyder, 1981). Since VIP has no effect on [^{3}H] spiperone-specific binding in the dorsal hippocampus obtained with unlabeled mianserin, it suggests that VIP acts primarily on 5-HT_1 receptor-binding sites. The fact that [^{3}H]spiperone binding is not affected also suggests that VIP does not modify the binding of spiperone to putative dopamine receptors in the hippocampus (Bischoff et al., 1979) or to butyrophenone-binding sites (Palacios et al., 1981). Finally, the observation that [^{3}H] 5-HT binding in the dorsal subiculum is not displaced by unlabeled spiperone (Deshmukh et al., 1982) strongly supports the hypothesis that VIP affects binding of [^{3}H] 5-HT to 5-HT_1 receptors in this structure.

Calcium ions have been shown to increase both specific binding of [^{3}H]5-HT and [^{125}I]VIP to brain membranes (Bennett and Snyder, 1976; Nelson et al., 1978; Taylor and Pert, 1979; Mallat and Hamon, 1982). Our data confirm those of Mallat and Hamon (1982) showing that increasing $CaCl_2$ concentrations in the incubation medium induces a dramatic increase in the number of 5-HT high affinity binding sites and a slight enhancement of their affiity for the labeled ligand. However, the efficacy of VIP to induce both changes in K_D and B_{max} for 5-HT_1-specific binding is not significantly affected by calcium. This effect of calcium might be the consequence of a modification of membrane viscosity. Heron et al. (1980) observed that fatty acids were able to increase both membrane viscosity and high affinity binding sites for [^{3}H]5-HT. Such a change in lipid membrane properties might also explain the alteration of their affinity for [^{3}H]5-HT binding caused by bacitracin, a peptidergic antibiotic. However, more work is needed to clarify this point. The increase of 5-HT_1-binding sites induced by VIP in the dorsal hippocampus is observed only in the presence of bacitracin; this can be related to a previous observation showing that bacitracin prevents the degradation of VIP and glucagon by liver membranes (Desbuquois et al., 1973; Bataille et al., 1974).

The dorsal hippocampus receives a large 5-HT innervation (Azmitia and Marovitz, 1980; Köhler et al., 1981) and contains high amounts of 5-HT and VIP receptors (Taylor and Pert, 1979; Biegon et al., 1982). Both 5-HT and VIP have been shown to influence electrical activity in this brain region (Dodd et al., 1979; Jahnsen, 1980; Segal, 1980). It has been recently observed that VIP may have some hypnogenic properties in rats by increasing paradoxical sleep duration; moreover, VIP can counteract insomnia induced by parachlorophenylalanine treatment (Riou et al., 1981). It may be suggested that the modulation by 5-HT of sleep-wakefulness states (Jacobs and Jones, 1978) can be influenced by VIP.

Finally, the subiculum, where the interaction between the VIP and 5-HT receptors has been visualized by quantitative autoradiography (Rostene et al., 1983), has been shown to be an important relay structure which collects information coming from other parts of the hippocampus and sends it to other brain regions (Meibach and Siegel, 1977). In view of this, the present data suggest that VIP effects through the 5-HT_1 receptor may be of special significance for the hippocampal influence on the rest of the brain.

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