ELSEVIER

European Journal of Pharmacology 405 (2000) 73–88

ejp

www.elsevier.nl/locate/ejphar

Spatial, cellular and temporal basis of vasopressin potentiation of norepinephrine-induced cAMP formation

Roberta Diaz Brinton [a,b,*], Richard H. Thompson [b], Elizabeth A. Brownson [b]

[a] *Department of Molecular Pharmacology and Toxicology, University of Southern California, Pharmaceutical Sciences Center, 1985 Zonal Avenue, Los Angeles, CA 90089 USA*

[b] *Program in Neuroscience, University of Southern California, Pharmaceutical Sciences Center, Los Angeles, CA 90089, USA*

Accepted 28 June 2000

Abstract

This study investigated the spatial distribution of vasopressin V_1 and β_1-adrenoceptors within hippocampal subfields and lamina in an attempt to localize the site(s) of interaction between these two receptor systems. In addition, the cell types, neuronal and glial, in which the vasopressin-induced neuromodulation occurs, were identified. Lastly, the temporal constraints of the potentiation induced by vasopressin were investigated. Results of these analyses demonstrated multiple sites within the hippocampus where the interaction between vasopressin and norephinephrine could occur. Moreover, vasopressin-induced potentiation of adrenergic stimulated cyclase occurred in both hippocampal neurons and glia whereas it did not occur in undifferentiated neurons. Analysis of the temporal constraints of vasopressin-induced potentiation revealed that pre-activation of the vasopressin V_1 receptor for 1 min yielded greater potentiation than simultaneous exposure to vasopressin and norepinephrine. These data provide insights into the spatial and temporal characteristics for the interaction between the vasopressin receptor and adrenoceptor systems and provide a cellular and biochemical rationale for the behavioral findings of Kovács and De Wied.

Keywords: Vasopressin; Norepinephrine; Neuromodulation; Associative memory mechanism; Hippocampus

1. Introduction

The pioneering research of De Wied et al. revealed that the neuropeptide, arginine vasopressin (AVP) could enhance memory function (De Wied, 1971, 1997). Efforts by Kovács and De Wied to elucidate the mechanism of vasopressin enhancement of memory function demonstrated a requirement of adrenergic innervation to the hippocampus (Kovács et al., 1979a,b). These investigators found that lesions of the dorsal noradreneregic bundle, which innervates the hippocampus (Moore and Bloom, 1979), abolished the behavioral effects of vasopressin (Kovács et al. 1979b). This study provided the first evidence that vasopressin interacted with norepinephrine to produce its behavioral effects. We pursued the biochemical basis for the dependency of vasopressin upon norepinephrine and found that vasopressin significantly potentiated norepinephrine-induced cAMP formation (Brinton and McEwen, 1989). Further results of this study demonstrated that vasopressin-induced potentiation was selective for β-adrenoceptor-stimulated adenylate cyclase (Brinton and McEwen, 1989). Several other interesting features of vasopressin-induced neuromodulation emerged from this investigation. First, in addition to the effect being dose-dependent and peptide specific, we also found that vasopressin-induced potentiation was blocked by a vasopressin V_1 receptor antagonist suggesting a Ca^{2+}-linked pathway. This postulate was supported by data demonstrating that vasopressin-induced potentiation was a Ca^{2+}-dependent process and could be blocked by an antagonist to the calcium-binding protein, calmodulin. In a later study, we documented that vasopressin V_1 receptor activation induced a rise in intracellular Ca^{2+} through activation of

* Corresponding author. Department of Molecular Pharmacology and Toxicology, University of Southern California, Pharmaceutical Sciences Center, 1985 Zonal Avenue, Los Angeles, CA 90089, USA. Tel.: +1-323-442-1430; fax: +1-323-442-1489.

E-mail address: rbrinton@hsc.usc.edu (R.D. Brinton).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00543-4

both the phosphatidyinositol signaling pathway and L-type Ca^{2+} channels (Brinton et al. 1994; Son and Brinton, 1998). These data suggest that vasopressin-induced potentiation is mediated by interaction with Ca^{2+}/calmodulin regulated adenylate cyclase, AC1, which is neurospecific and expressed in hippocampus as well as other select regions of the brain (Xia and Storm, 1997). Calcium/calmodulin regulated adenylate cyclase is of particular interest because of its importance for synaptic plasticity and learning and memory (Xia and Storm, 1997).

If vasopressin potentiation of norepinephrine-induced cAMP formation is the result of a direct interaction and not the result of a third intermediate component, the receptor systems for these two neurotransmitter systems would have to be spatially proximate. Results from two studies strongly suggested that receptors for vasopressin and norepinephrine are either functionally or structurally coupled in select regions of the hippocampus. Treatment of postnatal animals with high concentrations of vasopressin resulted in a down regulation of norepinephrine-induced cAMP formation without a diminution in the magnitude of the vasopressin-induced potentiation (Brinton, 1998). The total amount of cAMP generated was decreased but the relative magnitude of the potentiation was the same. Thus, it appeared that the vasopressin treatment had down-regulated the adrenergic response which suggested that a vasopressin V_1 receptor and β_1-adrenoceptor complex might exist. Results from a second independent study indicated that lesions of the dorsal noradrenergic bundle resulted in a site-specific upregulation of vasopressin receptors following lesioning of the dorsal noradrenergic bundle (Brinton, 1998). The continued presence of vasopressin receptors following the dorsal noradrenergic bundle lesion indicated that some, if not all, of the vasopressin receptors were postsynaptic to noradrenergic terminals. Secondly and surprisingly, the loss of adrenergic input to the hippocampus resulted in an upregulation of the vasopressin receptor system that modulates adrenergic stimulated cyclase. Taken together, results of these two studies suggested the intriguing possibility that a subpopulation of vasopressin and β-adrenoceptors exist as an adaptive and interactive complex. Thus, when an adaptive response occurs in one a correspondingly compensatory response occurs in the other.

The current study sought to expand our understanding of the spatial, cellular and temporal features of the interaction between vasopressin and norepinephrine. The first issue to be addressed in this study was the cytoarchitectural distribution of vasopressin and β-adrenoceptors in an attempt to localize the site(s) where an interaction between these two receptor systems was likely to occur. The study also sought to determine in which cell types, neuronal or glial, the neuromodulation occurred. Lastly, the temporal constraints of the potentiation induced by vasopressin were investigated. Results of these analyses demonstrated multiple sites within the hippocampus where the interaction between vasopressin and norephinephrine could occur and that vasopressin-induced potentiation of adrenergic stimulated cyclase occurred in both neurons and glia. Moreover, vasopressin potentiation of adrenergic stimulated cyclase is a property of differentiated cells and did not occur in nondifferentiated cells. Lastly, analysis of the temporal constraints of vasopressin-induced potentiation revealed that pre-activation of the vasopressin V_1 receptor within a circumscribed time window yielded greater potentiation than simultaneous exposure to vasopressin and norepinephrine. These data suggest that an accumulation of intracellular Ca^{2+} via vasopressin V_1 receptor activation prior to norepinephrine activation of β_1-adrenoceptors can substantially increase the magnitude of the potentiation leading to an increased generation of cAMP.

2. Material and methods

2.1. Vasopressin V_1 receptor autoradiography

Adult male Sprague–Dawley rats (Simonsen Labs. Gilroy, CA, $n = 3$) were decapitated and the brains rapidly removed and frozen on dry ice. Series or 15-μm-thick sections were mounted on chromalum gelatin-coated slides dried and stored at −80°C until use.

Autoradiographic labeling was performed as described by Chen et al. (1993). Briefly, sections were preincubated for 20 min in 50 mM Tris–HCl (pH 7.4), 100 mM NaCl, then rinsed twice for 5 min in 50 mM Tris–HCl at 22°C. Incubation was carried out for 60 min at 22°C by covering each section with 100–200 μl of incubation medium (50 mM Tris–HCl, 0.1 mM bacitracin, 5 mM $MgCl_2$, 1 mg/ml bovine serum albumin, pH 7.4) containing 10 nM [^{3}H]d(CH_2)$_5$Tyr(Me)AVP (NEN, Dupont) alone or in the presence of 10 μM arginine vasopressin. Incubation was followed by two 2-min washes in ice-cold incubation medium and a quick rinse in ice-cold distilled water. The slides were then dried in a stream of cold air, stored desiccated overnight at 0°C, then placed in a cassette apposed to tritium-sensitive Hyperfilm (Amersham) along with tritium-sensitive standards (Amersham) for 1 month at room temperature. Films were developed for 5 min in Kodak D19 and the sections stained with cresyl violet. Autoradiographic images were quantitatively analyzed using a standard curve generated from tritium standards with densitometric determinations performed using a BioQuant Autoradiography Image Analysis System. Specific binding ranged from 37% to 52% of the total binding. The use of 10 nM [^{3}H]d(CH_2)$_5$Tyr(Me)AVP to label V_1 receptors was based on several criteria of specificity. First, the pA_2 value for the V_1 antagonist for vasopresser activity was 8.62, which converts to a K_d value of 2.5 nM, whereas the pA_2 value for the V_1 receptor antagonist for antioxytocic activity was 6.62, which converts to a K_d value of 239 nM (Kruszynski et al., 1980). Thus, the antioxytocic effect

of the V_1 receptor antagonist used in the present study has an approximately 100-fold lower affinity for oxytocin receptors. Based on the pA_2 values and corresponding K_d values, 10 nM V_1 receptor antagonist would label 80% of the total number of V_1 receptors and 4% of the total number of oxytocin receptors. However, because of the very low affinity (239 nM) of the oxytocin receptors for the V_1 receptor antagonist, the antagonist is very likely to dissociate from the 4% fraction of the labeled oxytocin receptors during the post-labeling wash procedure.

In addition, the low-affinity antioxytocic effects are most apparent in the absence of Mg^{2+} and are significantly reduced in the presence of 0.5 mM Mg^{2+} (Kruszynski et al. 1980). The concentration of Mg^{2+} was 5 mM in the autoradiographic binding incubation buffer which is greater than that necessary to reduce the antioxytocic effects. Based on the 100-fold greater affinity of the V_1 receptor antagonist for V_1 receptors and the negative regulation of antioxytocic effects by Mg^{2+}, the labeling observed represents little to no labeling of oxytocin receptors.

2.2. Norepinephrine receptor autoradiography

Localization of β_1-adrenoceptors was determined in the same hippocampal sections used for vasopressin V_1 receptor localization. Control experiments to test for loss of β_1-adrenoceptors was achieved by performing [^{125}I]pindolol binding in rat brain sections at the time of [^{3}H]d-$(CH_2)_5$Tyr-(Me)AVP binding with those sections used as a reference point for assessing changes in [^{125}I]pindolol binding following storage at −80°C for 1 month. β_1-Adrenoceptors have been found to be the predominate subtype of adrenoceptor in the hippocampus (Minneman et al., 1981) and to be the adrenoceptor mediating both norepinephrine-induced cAMP accumulation in the hippocampus and norepinephrine effects upon long-term

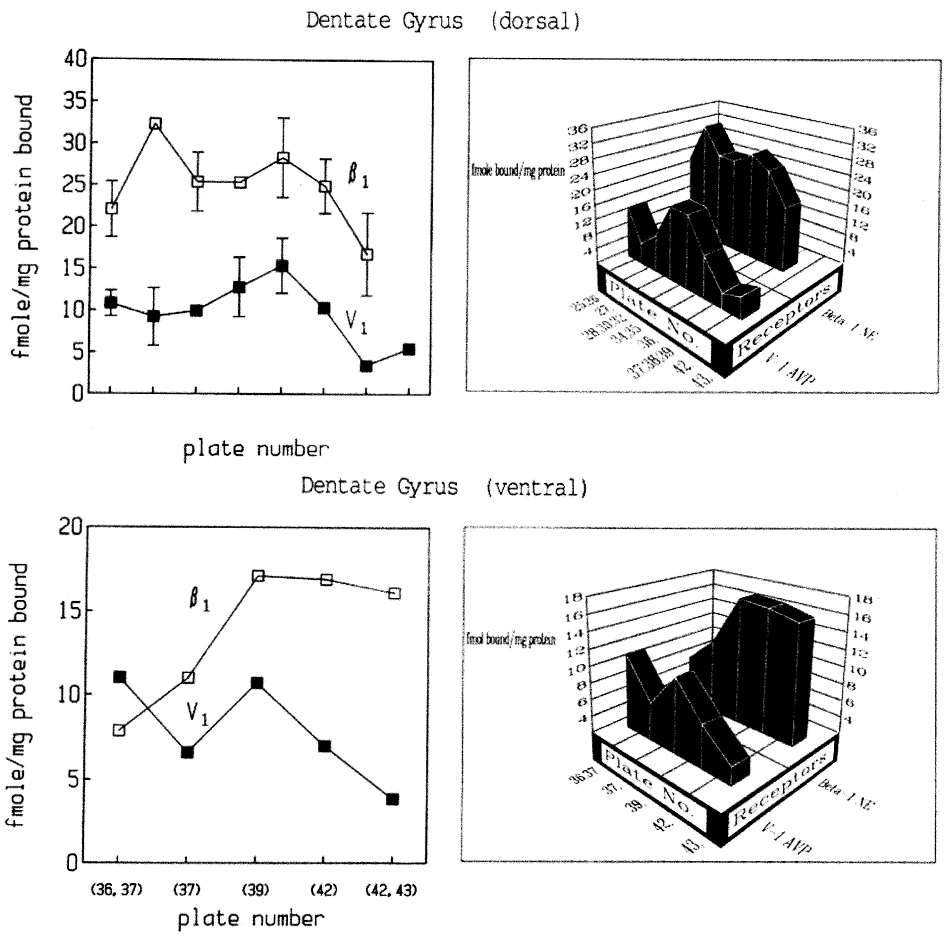

Fig. 1. Distribution of vasopressin V_1 receptors and β_1-adrenoceptors in the dorsal and ventral dentate gyrus. Receptor labeling was sequentially conducted in the same coronal section using [^{3}H]d$(CH_2)_5$Tyr(Me)AVP to label vasopressin V_1 receptors and [^{125}I]pindolol to label β_1-adrenoceptors. Data represent the mean ± SEM fmol/mg protein bound of [^{3}H]d$(CH_2)_5$Tyr(Me)AVP and [^{125}I]pindolol respectively derived from the densitometric analysis of three separate brain sections derived from three different rat brains ($n = 9$). Anatomical demarcations for analysis were based on cresyl violet stained coronal sections superimposed on autoradiographic images following the anatomical atlas of the rat brain by Paxinos and Watson (1986). Graphical representation in right panel provides the mean and SEM while the left panel provides a 3-D representation of the data that better illustrates the receptor density gradients that existed throughout the region depicted.

potentiation (Stanton and Sarvey, 1985) and importantly to be the adrenoceptor subtype potentiated by vasopressin (Brinton and McEwen, 1989). Thus, binding conditions utilized those found to be specific for β_1-adrenoceptor localization (Rainbow et al., 1984). β_1-Adrenoceptors were labeled using [^{125}I]iodopindolol (150 pM; 2200 Ci/mmol). Brain sections were incubated in 20 mM Tris–HCl, 135 mM $NaCl_2$ buffer, pH 7.4 at 23°C. For determination of β_1-adrenoceptors only, sections were incubated in 150 pM [^{125}I]pindolol (2200 Ci/mmol) in the presence of 50 nM ICI-118,551 to occupy β_2-adrenoceptors leaving β_1-adrenoceptors free for binding. L-propranolol (1 μM) was used to define nonspecific binding. The slices were apposed to film and exposed for 24 h. The contribution of the residual [^{3}H] to the [^{125}I] signal on film was negligible to none because of the very short exposure time necessary for iodinated compounds, maximum of 4 h for [^{125}I]pindolol. Exposure time of the film was based upon the cpm values from test slices wiped from the slide for liquid scintillation spectrometry.

2.3. Analysis of Autoradiograms:

Films were developed using standard developing procedures. Brain sections were stained with cresyl violet for histological comparison with autoradiographic images. Analysis of autoradiograms was accomplished using an IBM-based BioQuant System IV quantitative microdensitometry program equipped with a VBQ-Dage MTI 65 vidicon tube video camera, PC Vision Plus board, with video overlay interface, pseudocolor image enhancement and HIPAD digitizing tablet. Prior to analysis of norepinephrine/vasopressin receptor colocalization, nonspecific binding of the corresponding appropriate ligand was subtracted from the total binding. Quantitation of receptor density was based upon autoradiographic standards con-

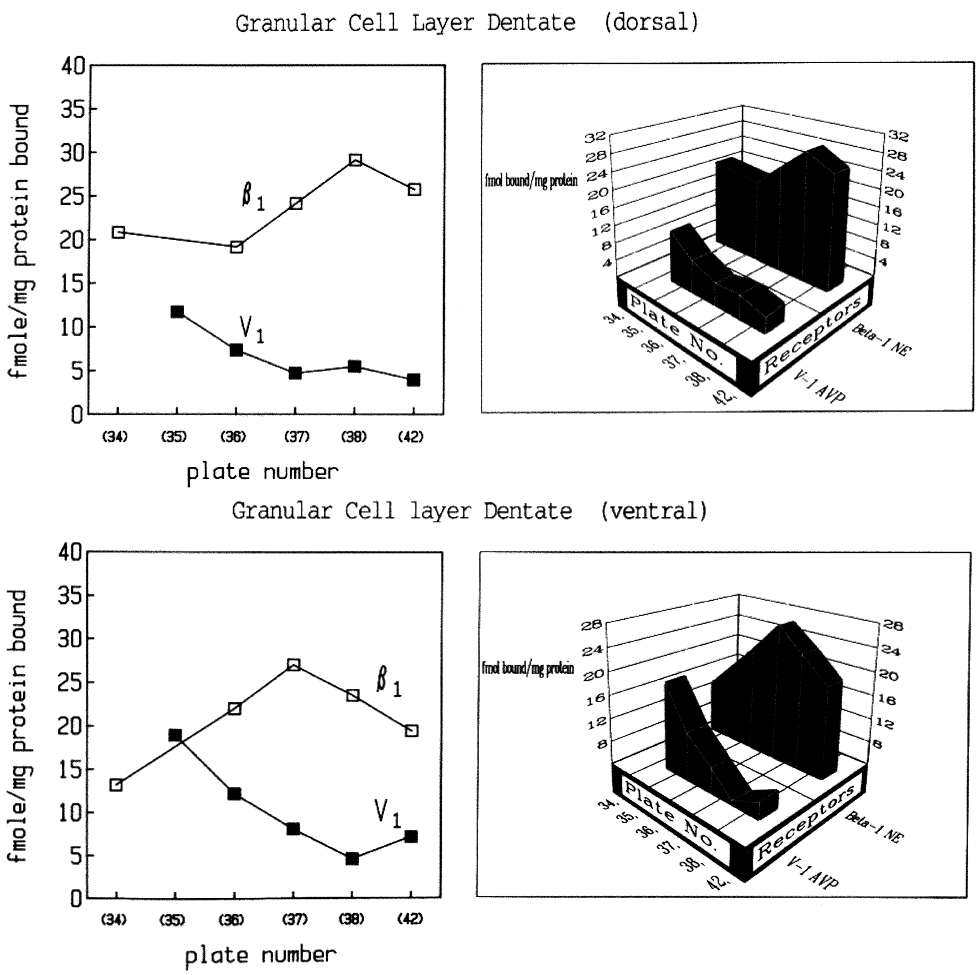

Fig. 2. Distribution of vasopressin V_1 receptors and β_1-adrenoceptors distribution in the dorsal and ventral granule cell layer of the dentate gyrus. Receptor labeling and analysis were the same as described in the legend to Fig. 1. Data represent fmol/mg protein bound of the [^{3}H]d(CH_2)$_5$Tyr(Me)AVP and [^{125}I]pindolol respectively derived from the densitometric analysis of three brain sections derived from one rat brain ($n = 3$). Graphical representation in right panel provides the receptor density while the left panel provides a 3-D representation of the data that better illustrates the receptor density gradients that existed throughout the region depicted.

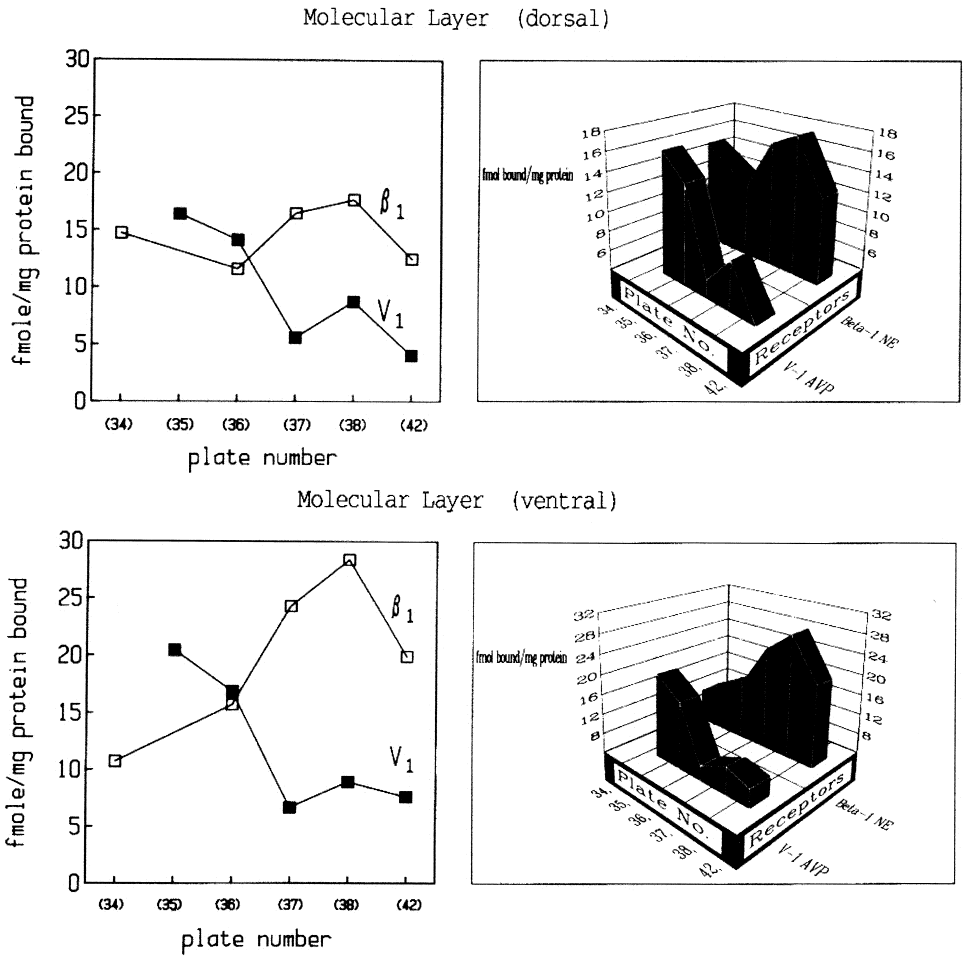

Fig. 3. Distribution of vasopressin V_1 receptors and β_1-adrenoceptors in the dorsal and ventral molecular layer of the dentate gyrus. Receptor labeling and analysis were the same as described in the legend to Fig. 1. Data represent fmol/mg protein bound of $[^3H]d(CH_2)_5Tyr(Me)AVP$ and $[^{125}I]$pindolol respectively derived from the densitometric analysis of three brain sections derived from one rat brain ($n = 3$). Graphical representation in right panel provides the receptor density while the left panel provides a 3-D representation of the data that better illustrates the receptor density gradients that existed throughout the region depicted.

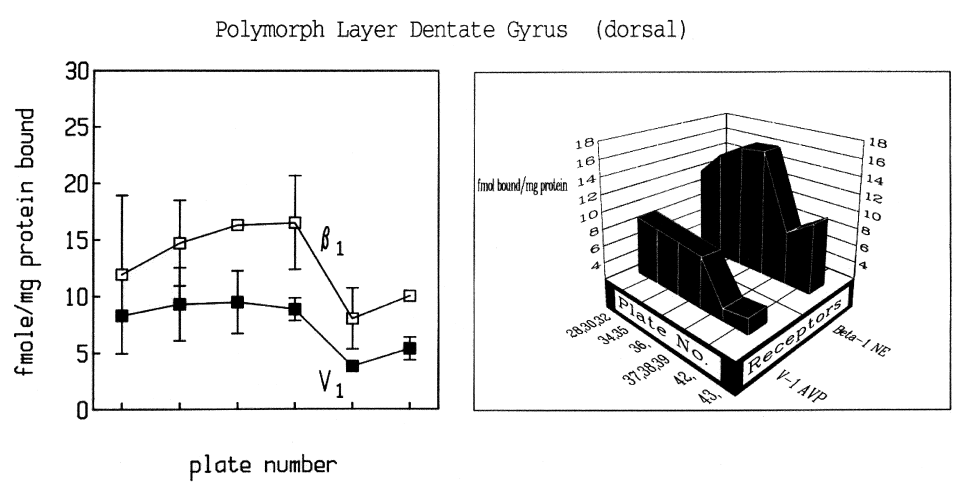

Fig. 4. Distribution of vasopressin V_1 receptors and β_1-adrenoceptors in the polymorph layer dentate gyrus. Receptor labeling and analysis were the same as described in the legend to Fig. 1. Data represent the mean ± SEM fmol/mg protein bound of $[^3H]d(CH_2)_5Tyr(Me)AVP$ and $[^{125}I]$pindolol respectively derived from the densitometric analysis of three separate brain sections derived from three different rat brains ($n = 9$). Graphical representation in right panel provides the mean and SEM while the left panel provides a 3-D representation of the data that better illustrates the receptor density gradients that existed throughout the region depicted.

taining known amounts of [^{3}H] and [^{125}I] (microscales available from Amersham). Co-localization of vasopressin V_1 and β_1-adrenoceptors to the same anatomic region in the hippocampus was accomplished by analyzing autoradiographic images labeled with [^{3}H]d(CH_2)$_5$Tyr(Me)AVP, storing that image as the reference and then superimposing autoradiographic images of [^{125}I]pindolol binding to determine areas of overlapping receptor labeling. Correlational statistics, Pearson *r* and statistical probability, were performed based on the areas which showed an overlap in receptor distribution.

2.4. Neuronal and glial cell culture

Primary neuron and glial cell culture preparation followed that described in Son and Brinton (1998) and Yamazaki et al. (1997). Neuronal cultures were allowed to grow for 14 days achieving morphological differentiation prior to use in biochemical experiments. Cultures of MC-XIV neuroblastoma cells followed the procedure described by Casper and Davies (1989). Enriched cultures of astroglia were obtained as described in Yamazaki et al. (1997).

2.5. cAMP determinations

Prior to exposure to test peptides, culture media was exchanged for Krebs Ringer (pH 7.4) containing: 124 mM NaCl, 5 mM KCl, 26 mM $NaHCO_3$ 1.3 mM $MgCl_2$, 1.4 mM KH_2PO_4, 0.8 mM $CaCl_2$, 10 mM dextrose and 1 mg/ml of 3-isobutyl-L-methylxanthine (IBMX; Sigma) as a phosphodiesterase inhibitor and incubated for 10 min at 37°C. Following the preincubation procedure, solution was exchanged for Krebs Ringer + IBMX + test substances. Test solutions containing 10 μM norepinephrine ([−]arterenol, Sigma) also contained 0.01% ascorbic acid.

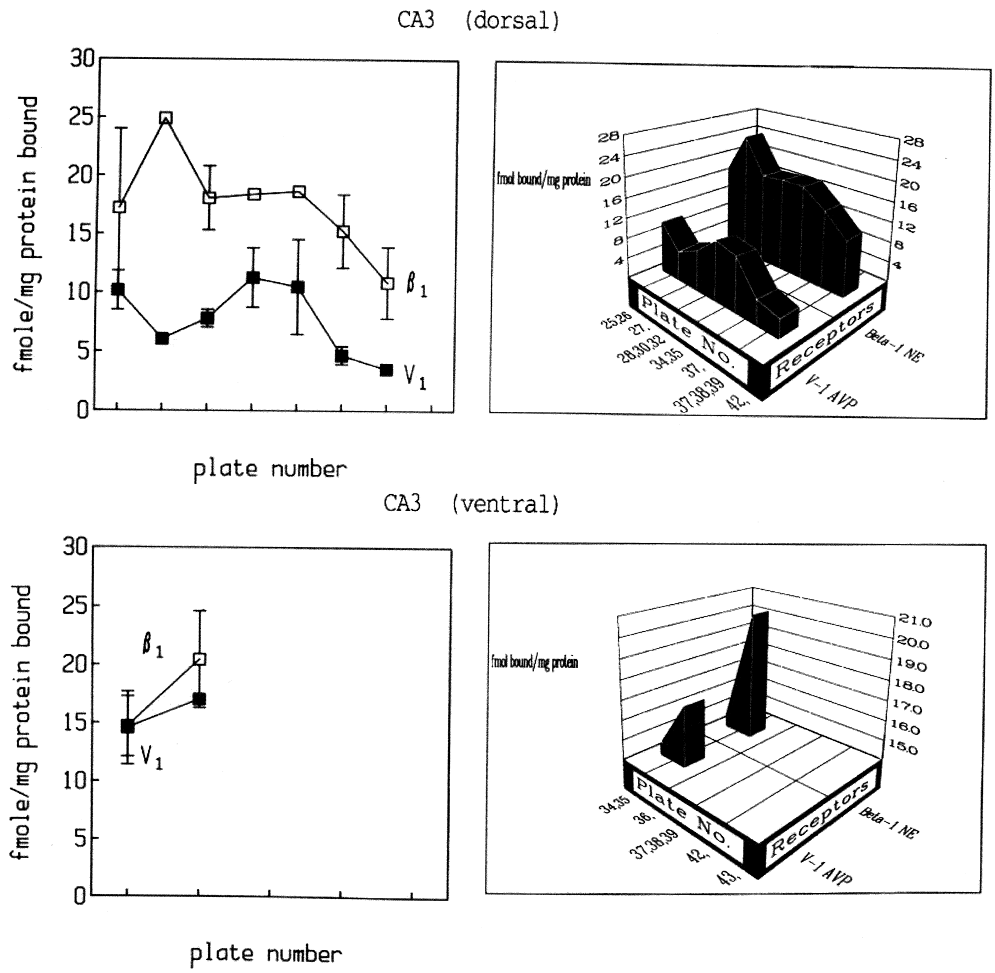

Fig. 5. Distribution of vasopressin V_1 receptors and β_1-adrenoceptors in the dorsal and ventral Cornue Ammon 3 (CA3). Receptor labeling and analysis were the same as described in the legend to Fig. 1. Data represent the mean ± SEM fmol/mg protein bound of [^{3}H]d(CH_2)$_5$Tyr(Me)AVP and [^{125}I]pindolol respectively derived from the densitometric analysis of three separate brain sections derived from three different rat brains ($n = 9$). Anatomical demarcations for analysis were based on cresyl violet stained coronal sections superimposed on autoradiographic images following the anatomical atlas of the rat brain by Paxinos and Watson (1986). Graphical representation in right panel provides the mean and SEM while the left panel provides a 3-D representation of the data that better illustrates the receptor density gradients that existed throughout the region depicted.

Vasopressin and [Phe^2, Ile^3, Orn^8]vasopressin (V_1 receptor agonist) were purchased from Peninsula Laboratories (Belmont, CA). All peptides were dissolved in Krebs solution immediately prior to use. Incubation at 37°C in the presence of test peptides continued for 15 min, unless stated otherwise. Termination of intracellular cAMP accumulation was accomplished by decantation of the test solution and immediate addition of 1 ml of ice-cold 7% trichloroacetic acid. Samples were then incubated for 45 min at 4°C. Trichloroacetic acid was transferred to glass test tubes and 1 ml of 0.2 N NaOH was added for the determination of protein concentration (Brinton and McEwen, 1989).

cAMP accumulation was determined using a protein kinase competitive binding assay previously described in Brinton and Brownson (1993). Experimental samples and cAMP standards dissolved in 7% trichloroacetic acid were extracted three times in six volumes of diethyl ether. Extracted samples (100 μl) and standards (100 μl) were incubated in at 4°C in the presence of 150 μl [5,8-^{3}H]adenosine-3′,5′-cyclic phosphate (Dupont, NEN) containing approximately 30,000 cpm/150 μl and 100 μl protein kinase A (Sigma) for 90 min. The binding reaction was terminated by the addition of 100 μl of activated charcoal in 1% BSA solution; the samples were then vortexed and centrifuged at $15{,}000 \times g$ for 10 min. Aliquots of supernatant (250 μl) were counted for radioactivity in a scintillation counter. cAMP content was determined by linear regression analysis based on a log/logit transformation. Data were analyzed either by a Student's *t*-test or by one-way analysis of variance followed by a Newman–Keuls test for multiple comparisons.

3. Results.

Spatial distribution of vasopressin V_1 receptors and β_1-adrenoceptors within the hippocampal subfields and lamina

3.1. General features of receptor distribution and abundance

Each of the subfields of the hippocampus expressed both vasopressin V_1 receptors and β_1-adrenoceptors. The distribution of vasopressin V_1 receptors and β_1-adrenoceptors within the hippocampus exhibited four important fea-

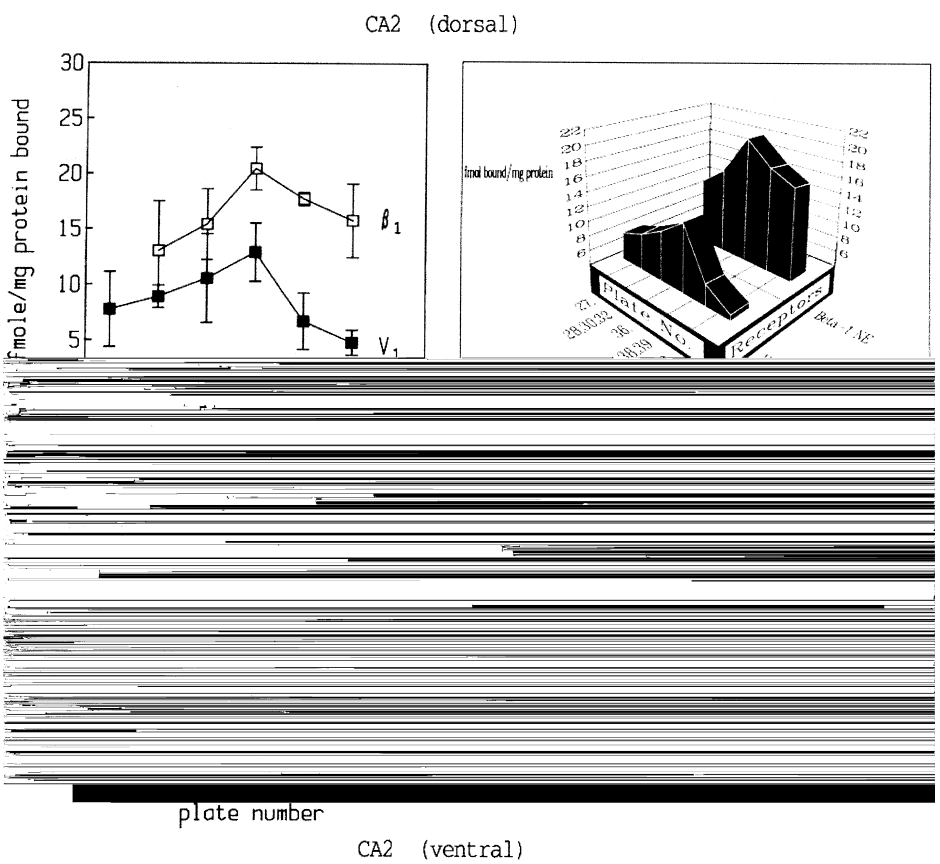

Fig. 6. Distribution of vasopressin V_1 receptors and β_1-adrenoceptors in the dorsal and ventral Cornue Ammon 2 (CA2). Receptor labeling and analysis were the same as described in the legend to Fig. 1. Data represent the mean ± SEM fmol/mg protein bound of [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP and [^{125}I]pindolol respectively derived from the densitometric analysis of three separate brain sections derived from three different rat brains ($n = 9$). Anatomical demarcations for analysis were based on cresyl violet stained coronal sections superimposed on autoradiographic images following the anatomical atlas of the rat brain by Paxinos and Watson (1986). Graphical representation in right panel provides the mean and SEM while the left panel provides a 3-D representation of the data that better illustrates the receptor density gradients that existed throughout the region depicted.

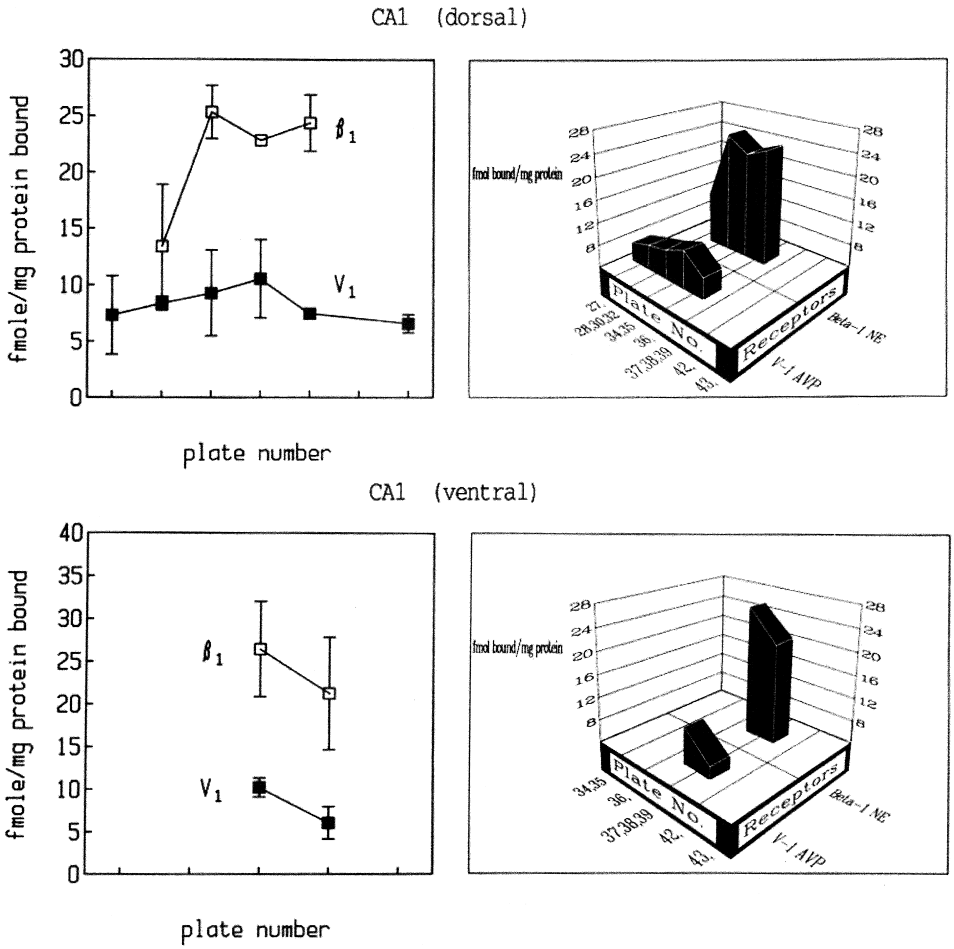

Fig. 7. Distribution of vasopressin V_1 receptors and β_1-adrenoceptors in the dorsal and ventral Cornue Ammon 1 (CA1). Receptor labeling and analysis were the same as described in the legend to Fig. 1. Data represent the mean ± SEM fmol/mg protein bound of [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP and [^{125}I]pindolol respectively derived from the densitometric analysis of three separate brain sections derived from three different rat brains ($n = 9$). Anatomical demarcations for analysis were based on cresyl violet stained coronal sections superimposed on autoradiographic images following the anatomical atlas of the rat brain by Paxinos and Watson (1986). Graphical representation in right panel provides the mean and SEM while the left panel provides a 3-D representation of the data that better illustrates the receptor density gradients that existed throughout the region depicted.

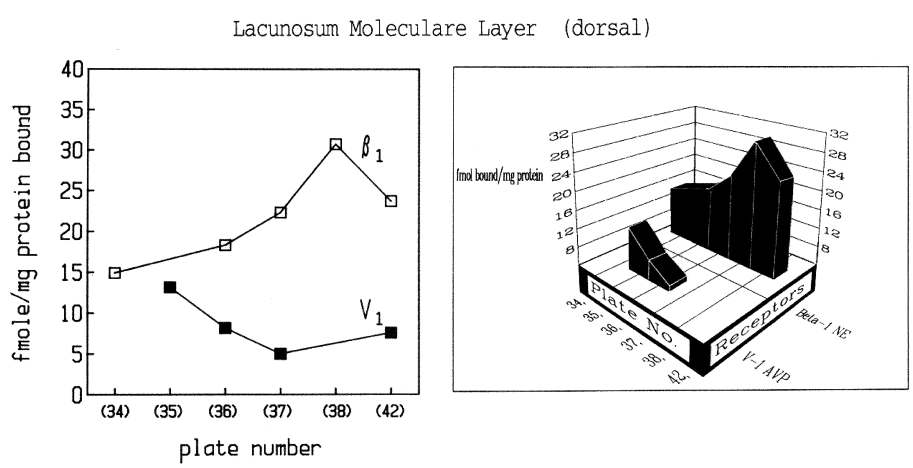

Fig. 8. Distribution of vasopressin V_1 receptors and β_1-adrenoceptors in the dorsal lacunosum molecular layer of CA1. Receptor labeling and analysis were the same as described in the legend to Fig. 1. Data represent fmol/mg protein bound of [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP and [^{125}I]pindolol respectively derived from the densitometric analysis of three brain sections derived from one rat brain ($n = 3$). Graphical representation in right panel provides the receptor density while the left panel provides a 3-D representation of the data that better illustrates the receptor density gradients that existed throughout the region depicted.

tures. First, the abundance of the vasopressin V_1 receptor was low relative to the β_1-adrenoceptor, although in some lamina the abundance of the vasopressin V_1 receptor was equal to that of the β_1-adrenoceptor. Second, despite the low abundance, the vasopressin V_1 receptor was consistently present in both the dorsal and ventral hippocampus, as was the β_1-adrenoceptor. Third, the distribution of vasopressin V_1 receptors exhibited a laminar specific expression. Fourth, within hippocampal subregions and within specific lamina, a rostral-caudal and dorsal-ventral gradient existed with the rostral and ventral regions generally exhibiting a greater abundance of vasopressin V_1 receptor. β_1-adrenoceptors were much more uniformly expressed throughout and did not show a pronounced gradient distribution.

3.2. Dentate gyrus

The dentate gyrus expressed a consistently high concentration of both vasopressin V_1 receptors and β_1-adrenoceptors (see Fig. 1). In the dorsal dentate gyrus, the expression of β_1 adrenoceptors was fairly uniform across the rostral caudal expanse. In contrast, vasopressin V_1 receptor expression was greatest in the mid-caudal portions of the dentate gyrus and was lowest at the more distal rostral and caudal sections. In the ventral dentate gyrus, the pattern of β_1-adrenoceptors and vasopressin V_1 receptors distribution was somewhat parallel to that expressed in the dorsal dentate with the exception that β_1 adrenoceptors were slightly lower at the rostral portion compared to more caudal sections.

A laminar analysis of vasopressin V_1 receptor and β_1-adrenoceptor distribution revealed remarkable distinctions in the patterns of expression. The dorsal and ventral portions of the granule cell layer showed a uniformly high level of β_1-adrenoceptor expression in both the rostral sections and caudal sections (Fig. 2). In contrast, vasopressin V_1 receptor expression was uniformly low and tapered to the lowest expression in the caudal sections (Fig. 2). In the molecular layer of the dentate gyrus, the

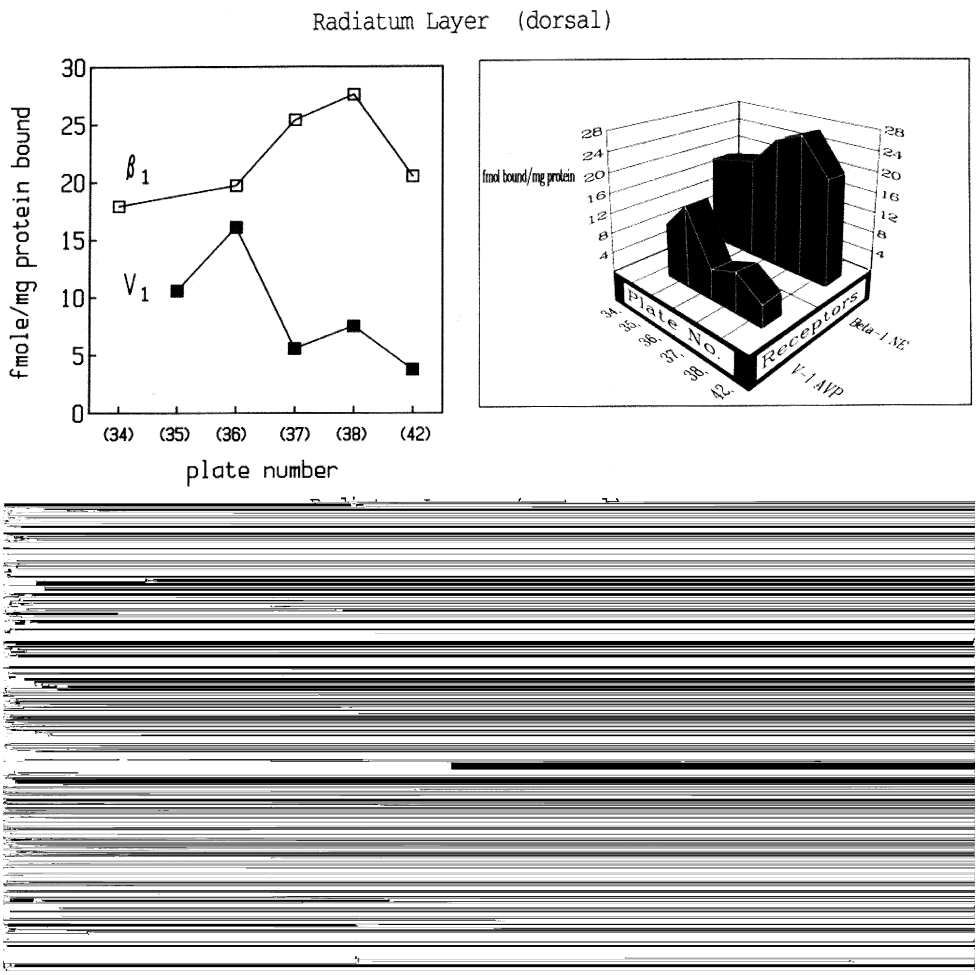

Fig. 9. Distribution of vasopressin V_1 receptors and β_1-adrenoceptors in the dorsal and ventral stratum radiatum of CA1. Receptor labeling and analysis were the same as described in the legend to Fig. 1. Data represent fmol/mg protein bound of [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP and [^{125}I]pindolol respectively derived from the densitometric analysis of three brain sections derived from one rat brain ($n = 3$). Graphical representation in right panel provides the receptor density while the left panel provides a 3-D representation of the data that better illustrates the receptor density gradients that existed throughout the region depicted.

same pattern as that of the granule cell layer appeared with the exception that both the dorsal and ventral portions exhibited an abundance of vasopressin V_1 receptors in the rostral sections that diminished in the caudal sections (Fig. 3).

The pattern of vasopressin V_1 receptor and β_1-adrenoceptor expression in the polymorph layer of the dentate gyrus showed parallel overlap between the two receptor systems. However, the density of vasopressin V_1 receptors was approximately half that of the β_1-adrenoceptors. Both receptors showed the greatest abundance of receptor expression in the rostral sections which declined in the caudal sections with the vasopressin V_1 receptors diminishing to very low expression in the caudal aspect (Fig. 4).

3.3. Cornue Ammon 3 (CA3)

The CA3 region of the hippocampus expressed vasopressin V_1 receptors and β_1-adrenoceptors in both the dorsal and ventral portions (Fig. 5). While the abundance of β_1-adrenoceptors exceeded that of vasopressin V_1 receptors, the pattern of expression for both receptor systems was parallel throughout the rostral to caudal extent of the CA3 region (Fig. 5).

3.4. Cornue Ammon 2 (CA2)

The CA2 region of the hippocampus expressed vasopressin V_1 receptors and β_1-adrenoceptors in both the dorsal and ventral portions (Fig. 6). The one exception to the abundance pattern of β_1-adrenoceptors occurred in the ventral portion of CA2 where the abundance of vasopressin V_1 receptors exceeded that of β_1-adrenoceptors. The overlap between the two receptors was greatest in the dorsal CA2 rostral sections (Fig. 6).

3.5. Cornue Ammon 1 (CA1)

The CA1 region of the hippocampus expressed a very low abundance of vasopressin V_1 receptors and a high concentration of β_1-adrenoceptors (see Fig. 7). In dorsal and ventral CA1, expression of β_1-adrenoceptors was fairly

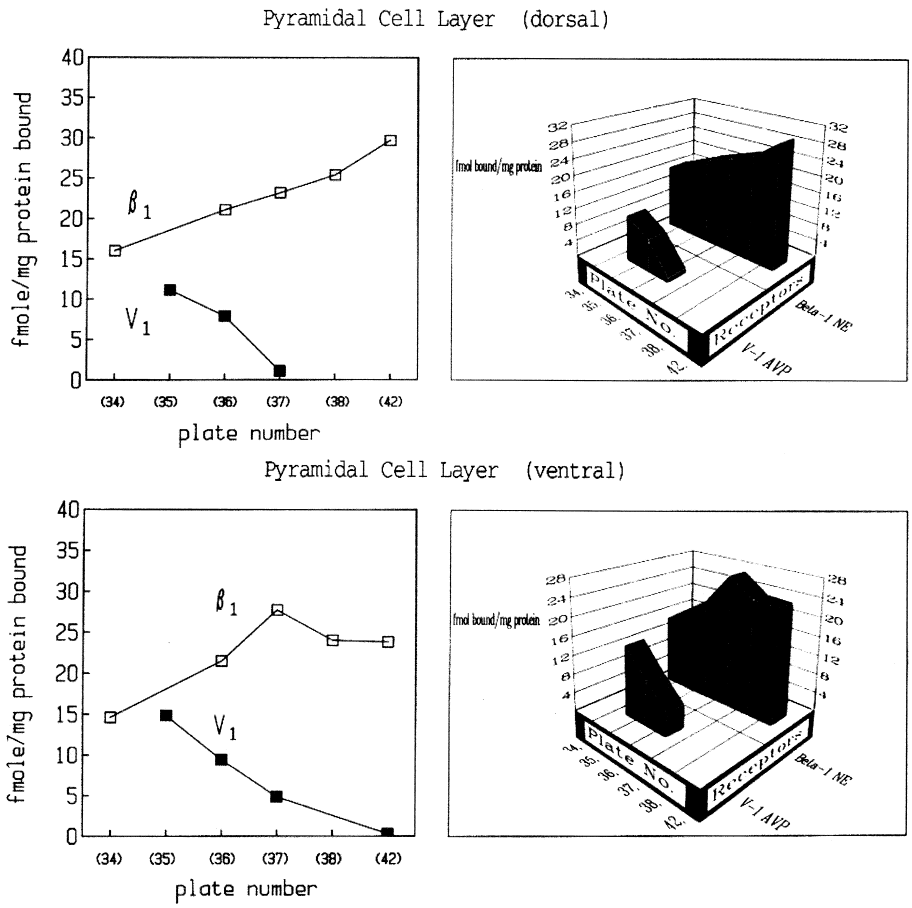

Fig. 10. Distribution of vasopressin V_1 receptors and β_1-adrenoceptors in the dorsal and ventral pyramidal cell layer of CA1. Receptor labeling and analysis were the same as described in the legend to Fig. 1. Data represent fmol/mg protein bound of [^{3}H]d(CH_2)$_5$Tyr(Me)AVP and [^{125}I]pindolol respectively derived from the densitometric analysis of three brain sections derived from one rat brain ($n = 3$). Graphical representation in right panel provides the receptor density while the left panel provides a 3-D representation of the data that better illustrates the receptor density gradients that existed throughout the region depicted.

uniform across the rostral to caudal expanse. The abundance of vasopressin V_1 receptor expression was consistently low throughout rostral and caudal sections. Overlap between the two receptor systems occurred throughout the rostral to caudal extent.

The laminar analysis of vasopressin V_1 receptor and β_1-adrenoceptor distributions in CA1 revealed remarkable distinctions in the pattern of vasopressin V_1 receptor and β_1-adrenoceptor expression. In the lacunosum molecular layer, a high abundance of β_1-adrenoceptors was present in the rostral and caudal sections (Fig. 8). Vasopressin V_1 receptors, which were expressed in low abundance, were most prevalent in the rostral sections and declined precipitously in the caudal aspects. In both the dorsal and ventral stratum radiatum, vasopressin V_1 receptor and β_1-adrenoceptor expression overlapped in the rostral portions; however, the density of vasopressin V_1 receptor dropped off precipitously in the caudal sections (Fig. 9). The pyramidal cell layer exhibited a pattern that was most striking in its dissociation between the two receptor systems (Fig. 10). β_1-adrenoceptor expression was high throughout the rostral and caudal aspects in both the dorsal and ventral regions. In contrast, vasopressin V_1 receptor expression occurred in the rostral sections and was completely absent in the caudal sections of the pyramidal cell layer. Expression of vasopressin V_1 receptors and β_1-adrenoceptors in the dorsal and ventral oriens layer were essentially parallel (Fig. 11). In the ventral oriens, there was a close parallel in both the sites and abundance of expression. In the ventral oriens, abundance of vasopressin V_1 receptors closely paralleled that of the β_1-adrenoceptors and were remarkably high throughout the rostral–caudal extent.

3.6. Cellular analysis of vasopressin potentiation of adrenoceptor-induced cAMP accumulation

Having determined the anatomical sites in the hippocampus where the interaction between vasopressin and norepinephrine was most likely to occur, we sought to determine the cell types that would contribute to the biochemical interaction between vasopressin and norepinephrine. In an attempt to determine whether the inter-

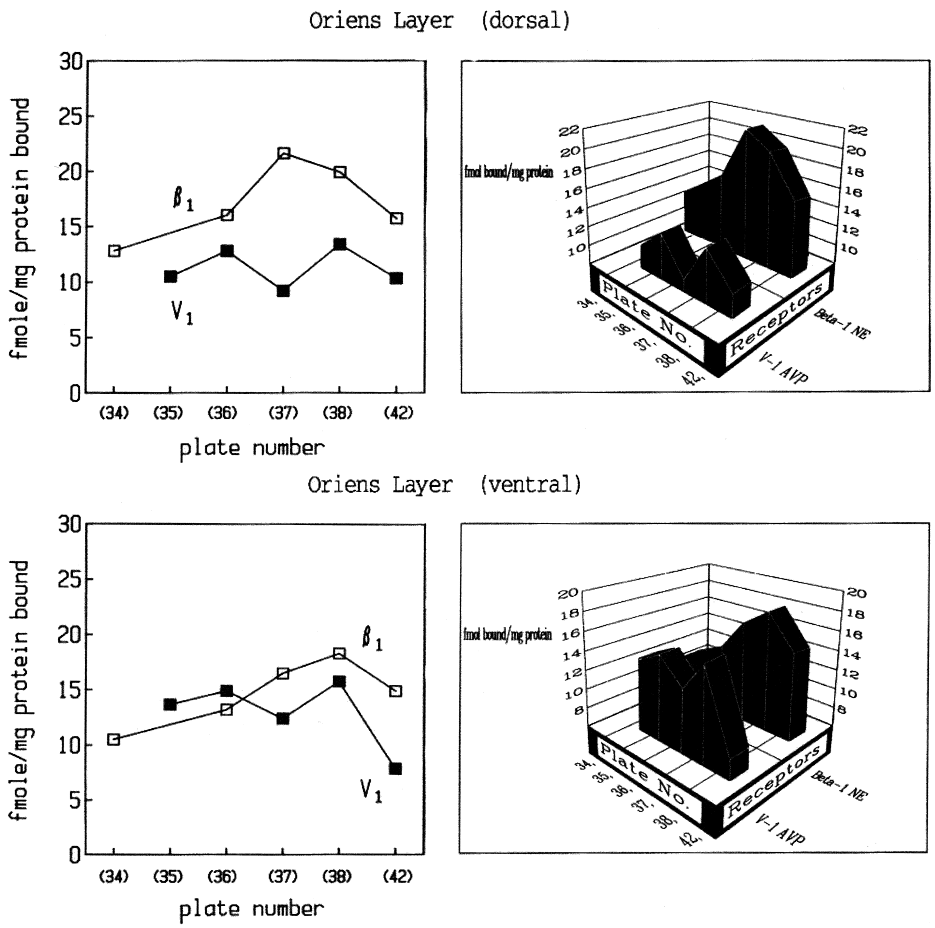

Fig. 11. Distribution of vasopressin V_1 receptors and β_1-adrenoceptors in the dorsal and ventral oriens layer of CA1. Receptor labeling and analysis were the same as described in the legend to Fig. 1. Data represent fmol/mg protein bound of [^{3}H]d(CH$_2$)$_5$Tyr(Me)AVP and [^{125}I]pindolol respectively derived from the densitometric analysis of three brain sections derived from one rat brain ($n = 3$). Graphical representation in right panel provides the receptor density while the left panel provides a 3-D representation of the data that better illustrates the receptor density gradients that existed throughout the region depicted.

action between vasopressin and norepinephrine was a feature of differentiated neurons, we investigated whether this interaction was expressed in undifferentiated neurons. In addition, we sought to determine the temporal constraints of the potentiation induced by vasopressin.

Results of the cellular analysis of vasopressin V_1 receptor agonist potentiation of norepinephrine-induced cAMP formation showed that both hippocampal neurons and hippocampal glial cells expressed the potentiation (Figs. 12 and 13). The magnitude of the potentiation, approximately a 25% increase above norepinephrine alone, was comparable in both cell types under the conditions where norepinephrine and vasopressin V_1 receptor agonist were added simultaneously. To determine whether the vasopressin-induced potentiation of norepinephrine-induced cAMP formation is a feature of only differentiated neurons, experiments were conducted using a neuroblastoma cell line, MC-XIC. Results of these experiments revealed that vasopressin alone induced cAMP formation that was significantly above baseline and that when norepinephrine and vasopressin were combined the result was additive (Fig. 14) and not synergistic as was the case in the differentiated neurons (Fig. 12).

3.7. Temporal analysis of vasopressin potentiation of adrenergic-induced cAMP accumulation

Earlier, we had found that activation of the vasopressin V_1 receptor in hippocampal neurons and glia activated the phosphatidyinositol signaling cascade which led to a sustained rise in intracellular calcium (Brinton et al., 1994; Son and Brinton, 1998). Because certain isoforms of adenylate cyclase are regulated by calcium/calmodulin

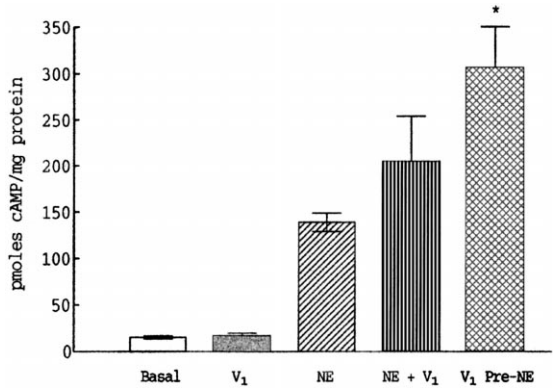

Fig. 13. Vasopressin V_1 receptor agonist potentiation of norepinephrine-induced cAMP formation in cultured hippocampal glial cells. Vasopressin V_1 receptor agonist (100 nM) exerted no effect on intracellular cAMP levels while norepinephrine (10 μM) increased intracellular cAMP fivefold above basal levels. Simultaneous addition of norepinephrine and vasopressin V_1 receptor agonist resulted in a 25% potentiation of norepinephrine-induced cAMP formation. Addition of the vasopressin V_1 receptor agonist 1 min prior to addition of norepinephrine significantly increased the potentiation above that of simultaneous addition of norepinephrine and vasopressin V_1 receptor agonist, ~35% increase, and increased the amount of intracellular cAMP to ~80% of that generated by norepinephrine alone. Data represent the mean ± SEM derived from one of three independent experiments, $n = 6$ per group. $^{**}P < .01$, $^{***}P < .001$.

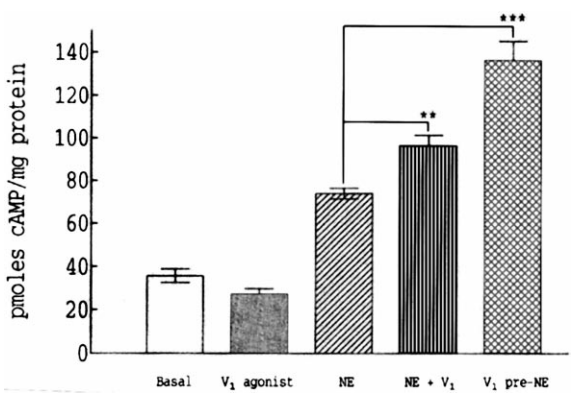

Fig. 12. Vasopressin V_1 receptor agonist potentiation of norepinephrine-induced cAMP formation in cultured hippocampal neurons. Vasopressin V_1 receptor agonist (100 nM) exerted no effect on intracellular cAMP levels while norepinephrine (10 μM) increased intracellular cAMP threefold above basal levels. Simultaneous addition of norepinephrine and vasopressin V_1 receptor agonist resulted in a significant (25%) potentiation of norepinephrine-induced cAMP formation. Addition of the vasopressin V_1 receptor agonist 1 min prior to addition of norepinephrine significantly increased the potentiation above that of simultaneous addition of norepinephrine and vasopressin V_1 receptor agonist, ~35% increase, and increased the amount of intracellular cAMP to ~80% of that generated by norepinephrine alone. Data represent the mean ± SEM derived from one of four independent experiments, $n = 6$ per group. $^{**}P < .01$, $^{***}P < .001$.

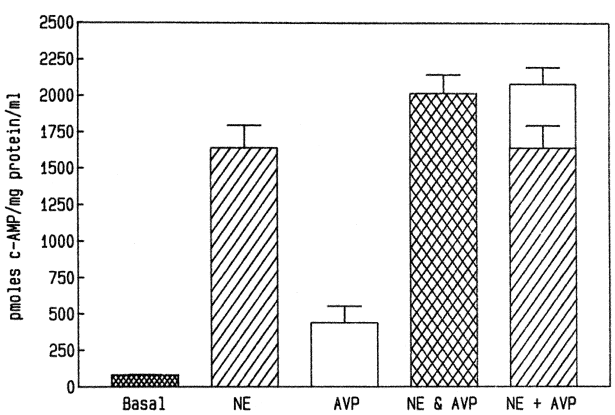

Fig. 14. Vasopressin induces cAMP directly in the MC-IXC neuroblastoma cell line which is additive to that induced by norepinephrine. MC-XIC neuroblastoma cells were exposed to 1 μM norepinephrine or 250 nM vasopressin (AVP) either alone or in combination. Norepinephrine induced a 10-fold increase in cAMP while AVP alone induced a threefold increase in intracellular cAMP. When added simultaneously, the level of intracellular cAMP was increased above that of norepinephrine alone; however, the increase was attributable to AVP-induction of cAMP directly as shown in the lat column, where the amount of cAMP induced by AVP alone was added to that induced by NE alone. The result showed an additive not synergistic effect. Data represent mean ± SEM derived from one of five independent experiments, $n = 6$ per group.

(Xia and Storm, 1997), we investigated whether preactivation of the V_1 receptor, thereby activating calcium signaling cascades prior to the activation of adenylate cyclase by norepinephrine, would impact the magnitude of the V_1 receptor agonist-induced potentiation. In the condition where V_1 receptor agonist was added 1 min prior to the addition of norepinephrine, the magnitude of the potentiation showed a marked increase in the potentiation. In neurons, the potentiation induced by V_1 receptor agonist increased from approximately 25% to approximately 60%. In glia, the increase in the potentiation was even more dramatic, from approximately 33% to 90% (fourth column Figs. 12 and 13).

An analysis of the temporal constraints for the increase magnitude of vasopressin-induced potentiation in hippocampal neurons was conducted. Results of this study revealed that simultaneous addition and a 30-s pre-exposure resulted in a 25% potentiation of norepinephrine-induced cAMP formation (Fig. 14). One minute of pre-exposure showed the greatest potentiation resulting in a 75% increase over norepinephrine alone (Fig. 15). The magnitude of the potentiation at 2- and 3-min pre-exposures was comparable to that which occurred under simultaneous exposure. A 10-min preexposure to V_1 receptor agonist was ineffective in potentiating norepinephrine-induced cAMP formation. These data provide evidence indicating that accumulation of intracellular calcium can profoundly affect the expression of and the magnitude of V_1 receptor agonist potentiation of norepinephrine-induced cAMP formation.

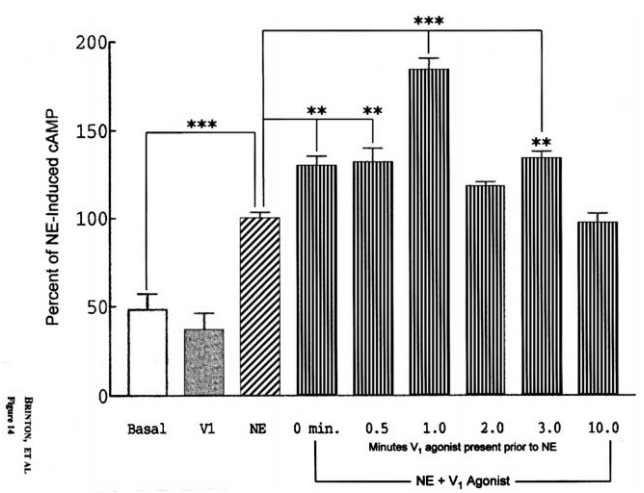

Fig. 15. Temporal profile of vasopressin V_1 receptor agonist potentiation of norepinephrine-induced cAMP formation in cultured hippocampal neurons. Simultaneous addition of vasopressin V_1 receptor agonist with norepinephrine induced a 25% potentiation. Pre-exposure of vasopressin V_1 receptor agonist at 30 s did not increase the degree of potentiation. Pre-exposure of vasopressin V_1 receptor agonist for 60 s resulted in a 75% potentiation of norepinephrine-induced cAMP formation. Longer pre-exposures were not as efficacious as the 1-min pre-exposure. Data represent mean ± SEM derived from one of three independent experiments, $n = 6$ per group. $^{**}P < 0.01$, $^{***}P < 0.001$.

4. Discussion

4.1. Spatial localization of vasopressin receptors and β-adrenoceptors

Each of the subfields of the hippocampus expressed both vasopressin V_1 receptors and β_1-adrenoceptors. The distribution of vasopressin V_1 receptors and β_1-adrenoceptors within the hippocampus exhibited four important features. First, the abundance of the vasopressin V_1 receptor was low relative to the β_1-adrenoceptor, although in some lamina the abundance of the vasopressin V_1 receptor was equal to that of the β_1-adrenoceptor. Second, despite the low abundance, the vasopressin V_1 receptor was consistently present in both the dorsal and ventral hippocampus, as was the β_1-adrenoceptor. Third, the distribution of vasopressin V_1 receptors exhibited a laminar-specific expression. Fourth, within the hippocampal subregions and within specific lamina, a rostral-to-caudal and dorsal-ventral gradient existed with the rostral and ventral regions generally exhibiting a greater abundance of vasopressin V_1 receptor. In contrast, β_1-adrenoceptors were much more uniformly expressed throughout and did not show pronounced gradient distribution.

Within the dentate gyrus, the spatial contiguity between the systems appeared to be the most prevalent. Given the receptor distributions, interaction between vasopressin V_1 receptors and β_1-adrenoceptors would be most likely to occur in the rostral portions of the molecular layer which is the dendritic zone of the dentate and receives input from the lateral entorhinal cortex and posterior CA1 (Raisman et al., 1965). The granule cell layer showed little promise for substantial interaction between the two systems. Most notable among the subfields was the polymorph layer of the dentate gyrus where vasopressin V_1 receptors and β_1-adrenoceptors showed a high degree of parallelism. The polymorph layer is innervated by axons of the septal area which could be, in part, vasopressinergic (Sofroniew, 1985). Interestingly, the dentate gyrus appears to be particularly responsive to vasopressin as vasopressin was found to induce long-term potentiation within the dentate gyrus in a Ca^{2+}-dependent manner (Chen et al., 1993).

The CA subfields showed parallel expression of both receptor systems. Surprisingly, the CA3 subfield showed the greatest degree of parallel expression suggesting that this site would be most likely to exhibit the interaction between β_1-adrenoceptor and vasopressin V_1 receptor signaling. A laminar analysis of the CA1 region demonstrated that the rostral portions of the dorsal and ventral radiatum and the ventral portion of the oriens layer were most likely to exhibit the interaction between vasopressin V_1 receptor and β_1-adrenoceptor signaling. The radiatum receives commissural and septal input with the vasopressin input most likely coming from the septal region (Sofroniew, 1985). The oriens layer receives input from the entorhinal region and the septal region (Sofroniew, 1985). It is no-

table that the layers that receive septal input appear to have the greatest likelihood of expressing the vasopressin potentiation.

In general, the cell body layers of the dentate gyrus and CA1 were unlikely to be the sites of interaction between vasopressin V_1 receptor and β_1-adrenoceptor signaling because of the low abundance of vasopressin V_1 receptor expression. Instead, the sites most likely, based on the receptor localization, were dendritic zones of the molecular layer of the dentate, especially the polymorph layer of the dentate, and the radiatum and oriens layer of CA subfields.

4.2. Cellular specificity and temporal constraints

Vasopressin-induced potentiation of β_1-adrenoceptor-induced cAMP formation occurred in differentiated hippocampal neurons whereas it did not occur in undifferentiated neurons, the neuroblastoma cells. These data indicate that the interaction between the vasopressin V_1 receptor and β_1 adrenoceptors is a feature of differentiated neurons as the vasopressin-induced potentiation was not present in the neuroblastoma cells but was present in the primary cultured hippocampal neurons that were morphologically differentiated. These data are consistent with a developmentally regulated vasopressin induction of cAMP formation in undifferentiated cultured hippocampal neurons that declined and then fully disappeared upon morphological differentiation (Brinton and Brownson, 1993).

Remarkably, vasopressin-induced potentiation of β_1-adrenoceptor-induced cAMP formation also occurred in glial cells. The functional significance of this finding is not yet fully understood; however, the findings of Yamazaki et al. demonstrated the presence of vasopressin V_1 receptor mRNA in neurons and glia (Yamazaki et al., 1997). Importantly, biochemical and molecular studies have shown that the vasopressin V_1 receptors are functional in both neurons and glia (Brinton et al., 1994; Brinton et al., 1998; Zhao and Brinton, 2000).

Investigation of the temporal constraints of vasopressin-induced potentiation of β_1-adrenergic-induced cAMP formation yielded interesting results. The data show that in both neurons and in glia, preactivation of the vasopressin V_1 receptor led to an increase in intracellular Ca^{2+}, which markedly increased the magnitude of the potentiation (Son and Brinton, 1998). Pre-activation for 1 min produced the greatest potentiation. Simultaneous activation of both vasopressin V_1 receptors and β_1-adrenoceptors or time frames less than or greater than 1 min were not as effective. Interestingly, the temporal coding for this effect is consistent with the principles of associative learning (Thompson et al., 1997)

4.3. Functional implications

Data from this study provide insights on several issues. First, the autoradiographic data show that the potential for the interaction between vasopressin and norepinephrine exists throughout the hippocampus. Moreover, the interaction between the systems can occur both in neurons and glia. These data would suggest three functional corollaries. First, the properties necessary for vasopressin potentiation of adrenergic stimulated cyclase are in place throughout the hippocampus, in varying degrees, and in multiple cell types. Second, because the potential for interaction is widely dispersed, it would appear that the synaptic wiring of vasopressinergic or adrenergic presynaptic terminals is the mechanism that confers specificity for the sites of interaction between these two neurotransmitter systems. Based on the autoradiographic and cellular data, it would appear that the system is constructed in such a way that allows for the development of experience-dependent synaptic configurations that once in place can activate the potentiating interaction between vasopressin and norephinephrine effect or mechanisms. Third, the data also suggest that the capacity for interacting biochemical systems is one of the factors that defines the differentiated state of cellular development. Consistent with that postulate is the suggestion that associative biochemical mechanisms that could underlie associative learning and memory are defining characteristics of the differentiated state of cellular development.

The question that emerges from this work is: What purpose does neuromodulation serve? A simple, yet compelling hypothesis is that associative learning processes are dependent upon associative biochemical events (Brinton, 1990). In this light, associative and conditional neuromodulation may well serve as the biochemical analog for the processes that underlie associative learning and memory which are behaviorally manifested as an enhancement of memory function. The complexity and conditionality of neuromodulation greatly expands the signaling and information coding possibilities of neural networks (Brinton, 1990). This postulate has been supported over the years from biochemical and molecular analyses that show that synergistic activation of adenylate cyclase by Ca^{2+}/calmodulin-dependent mechanisms leads to an enhanced activation of transcription factors that activate genes important for long-term memory function (Xia and Storm, 1997; Sheng et al,. 1991; Kasai and Petersen, 1994; Hille, 1992). It has now been well demonstrated that varying concentrations of intracellular second messengers can result in different patterns of gene expression (Berridge, 1998). Data contained within the present report provide evidence indicating that activation of a memory system prior to the activation of the arousal system induces a greater magnitude of the biochemical arousal response which could provide a basis for regulating gene expression necessary for long-term memory processes.

In conclusion, the pioneering findings of Kovács and De Wied (Kovács et al., 1979b), revealing a requirement of norepinephrine for the manifestation of the memory-enhancing effects of vasopressin, provided a critical behavioral foundation upon which to explore the broader signifi-

cance of interacting neurotransmitter systems. Moreover, this discovery laid the foundation for exploring the interaction between biochemical and molecular systems that underlie the behavioral expression of associative long-term memory function.

Acknowledgements

The discoveries made by Professor Dr. David De Wied and his colleagues have inspired and challenged many neuroscientists around the globe, myself among them. I, like many others, first became aware of Professor De Wied's research through the scientific literature. His ideas and discoveries established the cutting edge of the science at the time and he has been doing so ever since. This perception is not only supported by the scientific record but is also substantiated by the large number of scientists who began to work in the area following his discoveries, some sought to build upon those discoveries while others sought to refute his findings. Regardless of the motivation, it is a clear mark of compelling and interesting science when it sparks international attention, competition and rivalry. One of the many exceptional qualities that speaks to the character of Professor De Wied, is that as the competition grew so did his generosity and hospitality to the international community in his work. I was fortunate to be a beneficiary of this scientific hospitality when Dr. Ron DeKloet, then of the Rudolf Magnus Institute, invited me to the institute as a visiting scientist. The Rudolf Magnus Institute of Pharmacology, as it was known under De Wied's leadership, radiated the ideals of science: rigorous scientific thought, creative scholarly thinking, exacting execution of experimental paradigms, an international gathering of scientists and a passion for discovery. To achieve the ideals of science and to build an institute that embodies those ideals for many to experience and learn is a rare achievement. These extraordinary achievements are not easily or rapidly created. It takes a lifetime of commitment and dedication to that vision. The fruits of Professor De Wied's generosity of intellect and spirit are held in many of us — and in each of us his legacy of scientific rigor, of scientific scholarship, of scientific courage and fortitude has taken seed. Professor Dr. David De Wied is held in the greatest admiration and has the deepest gratitude of those of us who were fortunate enough to be given the gift of his scientific mentorship and friendship.

This work was supported by grants from the National Institutes of Health MH 46036 and the Norris Foundation to RDB.

References

Berridge, M.J., 1998. Neuronal calcium signaling. Neuron 21, 13–26.

Brinton, R.D., 1998. Vasopressin in the mammalian brain: the neurobiology of a mnemonic peptide. In: Urban, I.J.A., Burbach, J.P., de Wied, D. (Eds.), Progr. Brain Res. Elsevier, Dublin, Ireland, pp. 177–199.

Brinton, R.E., 1990. Neuromodulation: associative and nonlinear adaptation. Brain Res. Bull. 24, 651–658.

Brinton, R.D., Brownson, E.A., 1993. Vasopressin-induction of cyclic AMP in cultured hippocampal neurons. Dev. Brain Res. 71, 101–105.

Brinton, R.E., McEwen, B.S., 1989. Vasopressin neuromodulation in the hippocampus. J. Neurosci. 9, 752–759.

Brinton, R.D., Gonzalez, T.M., Cheung, W.S., 1994. Vasopressin-induced calcium signaling in cultured hippocampal neurons. Brain Res. 667, 151–159, [corrected and republished article originally printed in Brain Res., 661 (1–2) (Oct. 24, 1994) 274–282].

Brinton, R.D., Yamazaki, R., Gonzalez, C.M., O'Neill, K., Schreiber, S.S., 1998. Vasopressin-induction of the immediate early gene, NGFI-A, in cultured hippocampal glial cells. Mol. Brain Res. 57, 73–85.

Casper, D., Davies, P., 1989. Mechanism of activation of choline acetyltransferase in a human neuroblastoma cell line. Brain Res. 478, 85–94.

Chen, C., Diaz, B.R., Shors, T.J., Thompson, R.F., 1993. Vasopressin induction of long-lasting potentiation of synaptic transmission in the dentate gyrus. Hippocampus 3, 193–203.

De Wied, D., 1971. Long term effect of vasopressin on the maintenance of a conditioned avoidance response in rats. Nature 232, 58–60.

De Wied, D., 1997. Neuropeptides in learning and memory processes. Behav. Brain Res. 83, 83–90.

Hille, B., 1992. G protein-coupled mechanisms and nervous signaling. Neuron 9, 187–195.

Kasai, H., Petersen, O.H., 1994. Spatial dynamics of second messengers: IP3 and cAMP as long-range and associative messengers. Trends Neurosci. 17, 95–101.

Kovács, G.L., Bohus, B., Versteeg, D.H.G., 1979a. Facilitation of memory consolidation by vasopressin: mediation by terminals of the dorsal noradrenergic bundle? Brain Res. 172, 73–85.

Kovács, G.L., Bohus, B., Versteeg, D.H., de Kloet, E.R., de Wied, D., 1979b. Effect of oxytocin and vasopressin on memory consolidation: sites of action and catecholaminergic correlates after local microinjection into limbic-midbrain structures. Brain Res. 175, 303–314.

Kruszynski, M., Lammek, B., Manning, M., Seto, J., Haldar, J., Sawyer, W.H., 1980. [1-beta-Mercapto-beta,beta-cyclopentamethylenepropionic acid),2-(*O*-methyl)tyrosine argine-vasopressin and [1-beta-mercapto-beta,beta-cyclopentamethylenepropionic acid)]argine-vasopressine, two highly potent antagonists of the vasopressor response to arginine-vasopressin. J. Med. Chem. 23, 364–368.

Minneman, K.P., Pittman, R.N., Molinoff, P.B., 1981. Beta-adrenergic receptor subtypes: properties, distribution, and regulation. Annu. Rev. Neurosci. 4, 419–461.

Moore, R.Y., Bloom, F.E., 1979. Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. Annu. Rev. Neurosci. 2, 113–168.

Paxinos, G., Watson, C., 1986. The Rat Brain in Sterotaxic Coordinates. Academic Press, Orlando, FL.

Rainbow, T.C., Parsons, B., Wolfe, B.B., 1984. Quantitative autoradiography of beta 1- and beta 2-adrenergic receptors in rat brain. Proc. Natl. Acad. Sci. U. S. A. 81, 1585–1589.

Raisman, G., Cowan, W.M., Powell, T.P.S., 1965. The extrinsic afferent, commissural and association fibres of the hippocampus. Brain 88, 963–996.

Sheng, M., Thompson, M.A., Greenberg, M.E., 1991. CREB: a Ca(2+)-regulated transcription factor phosphorylated by calmodulin-dependent kinases. Science 252, 1427–1430.

Sofroniew, M.V., 1985. Vasopressin-and neurophysin-immunoreactive neurons in the septal region, medial amygdala and locus coeruleus in colchicine-treated rats. Neuroscience 15, 347–358.

Son, M.C., Brinton, R.D., 1998. Vasopressin-induced calcium signaling in cultured cortical neurons. Brain Res. 793, 244–254.

Stanton, P.K., Sarvey, J.M., 1985. Blockade of norepinephrine-induced long-lasting potentiation in the hippocampal dentate gyrus by an inhibitor of protein synthesis. Brain Res. 361, 276–283.

Thompson, R.F., Bao, S., Chen, L., Cipriano, B.D., Grethe, J.S., Kim,

J.J., Thompson, J.K., Tracy, J.A., Weninger, M.S., Krupa, D.J., 1997. Associative learning. Int. Rev. Neurobiol. 41, 151–189, [Review] [143 refs].

Xia, Z., Storm, D.R., 1997. Calmodulin-regulated adenylyl cyclases and neuromodulation. Curr. Opin. Neurobiol. 7, 391–396.

Yamazaki, R.S., Chen, Q., Schreiber, S.S., Brinton, R.D., 1997. Localization of V_1 vasopressin receptor mRNA expression in cultured neurons, astroglia, and oligodendroglia of rat cerebral cortex. Mol. Brain Res. 45, 138–140.

Zhao, L., Brinton, R.D., 2000. Vasopressin induced calcium signaling in cultured cortical astrocytes. Soc. Neurosci. Abstr. 3, 15546.