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Effects of Guanine Nucleotides on CNS Neuropeptide Receptors

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The effect of nucleotides on central nervous system neuropeptide receptor binding was investigated. The guanine nucleotides, guanosine-5′-triphosphate and guanylyl-5′-imidodiphosphate, significantly inhibited the binding of radiolabeled vasoactive intestinal polypeptide but not that of [Tyr^4]bombesin to rat brain membranes. Vasoactive intestinal polypeptide binding was inhibited by guanine nucleotides in a dose-dependent manner. Using a 20 μM dose, 60% of the specific vasoactive intestinal polypeptide binding was inhibited by guanylyl-5′-imidodiphosphate, which was more potent than guanosine-5′-triphosphate, whereas other nucleotides were not effective. This reduction in binding was a consequence of lower affinity of the receptor for vasoactive intestinal polypeptide, which in turn resulted from an increased rate of dissociation.

Key words: **bombesin receptors, guanine nucleotides, neuropeptide receptors, vasoactive intestinal polypeptide (VIP) receptors**

Bombesin (BN) and vasoactive intestinal polypeptide (VIP) represent two classes of neuropeptides active in the central nervous system (CNS) and gastrointestinal (GI) tract. Specific plasma membrane receptors for [Tyr^4]BN [1] and VIP [2] have been characterized using acinar cells dispersed from the guinea pig pancreas. In this cell line BN increases Ca^{2+} flux and elevates intracellular levels of cGMP after interaction with its receptor, whereas VIP increases cAMP levels and stimulates adenylate cyclase activity [3]. Similarly, specific synaptosomal receptors for [Tyr^4]BN [4] and VIP [5] have been characterized using rat brain membranes. Also, VIP increases cAMP levels in brain slices and stimulates adenylate cyclase activity in brain homogenate, whereas BN does not [6–8]. These data indicate that both peripheral and central VIP receptors may be positively coupled to adenylate cyclase, whereas BN receptors are not.

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The modulatory effects of guanosine-5′-triphosphate (GTP) and its relatively nonmetabolizable analog guanylyl-5′-imidodiphosphate (GMP-P(NH)P) on various hormone, neurotransmitter, and drug receptors coupled to adenylate cyclase has been well studied. These guanine nucleotides negatively modulate agonist binding to the glucagon [9], α-adrenergic [10], β-adrenergic [11, 12], dopamine [13], muscarinic cholinergic [14], and opiate receptors [15]. In addition, GTP regulates the functional coupling of glucagon [16, 17], β-adrenergic [16, 18], and dopamine [19, 20] receptors to adenylate cyclase. Thus guanine nucleotide triphosphates may interact with a plasma membrane regulatory subunit and alter receptor conformations such that receptor–hormone interaction with adenylate cyclase is affected and agonist dissociates more rapidly from receptor [21].

In this communication the effects of GMP-P(NH)P and other nucleotides on radiolabeled $[Tyr^4]$BN and VIP binding to CNS neuropeptide receptors are investigated. The observed negative modulatory effects of GMP-P(NH)P on VIP binding are compared to those previously obtained for other receptors coupled to adenylate cyclase.

MATERIALS AND METHODS

Crude rat brain homogenate was prepared as follows. Male Sprague–Dawley rats (150–200 gm) were decapitated and the brains dissected. The cerebellum and medulla/pons, which contained minimal neuropeptide binding activity, were removed. The remaining brain was homogenized in 100 volumes of 50 mM Tris-HCl (pH 7.4) at 4°C using a Brinkmann Polytron (setting 5, 15 sec). After centrifugation at 25,000g for 15 min the pellet was resuspended in 100 volumes of cold buffer. The homogenate was again centrifuged at 25,000g for 15 min, and the pellet resuspended in 10 volumes of buffer to yield a crude brain homogenate. The membranes were then assayed for $[Tyr^4]$BN and VIP binding activity.

The $[Tyr^4]$BN binding assay was performed using the protocol of Moody et al [4]. $[Tyr^4]$BN was monoiodinated such that the C-terminal methionine was not oxidized. Freshly prepared homogenate (200 μl, 1.6 mg protein) was incubated with 1 nM of $[^{125}I\text{-}Try^4]$BN at 4°C for 20 min in the presence or absence of competitor. The buffer contained 0.1% bovine serum albumin (Cohn fraction V, Sigma) in 50 mM Tris-HCl (pH 7.4). Bound peptide was separated from free using the centrifugation [1] or filtration [4] techniques described previously.

The VIP binding assay was performed using the protocol of Taylor and Pert [5]. VIP was monoiodinated using the procedure of Christophe et al [2]. Freshly prepared homogenate (100 μl) was incubated with 0.2 nM $[^{125}I]$VIP at 30°C for 20 min in the presence or absence of competitor. The buffer contained 1% bovine serum albumin and 5 mM $MgCl_2$ in 50 mM Tris-HCl (pH 7.4); $MgCl_2$ was observed to increase the specific binding. Bound peptide was separated from free using the sucrose density gradient centrifugation technique of Taylor and Pert [5]. This binding assay differs from $[Tyr^4]$BN binding assay because: (a) VIP is more hydrophobic than $[Tyr^4]$BN and (b) VIP equilibrates more slowly at 4°C with its receptor than $[Tyr^4]$BN; thus a higher temperature is required to accelerate the binding kinetics.

When neuropeptide binding studies were performed in the presence of nucleotides, membranes were preincubated with fresh nucleotide solution in 50 mM Tris-HCl (pH 7.4) for 10 min prior to the addition of radiolabeled peptide. AMP, ATP, CMP, CTP, GMP and UMP were from Sigma; all other nucleotides were purchased from Boehringer-Mannheim.

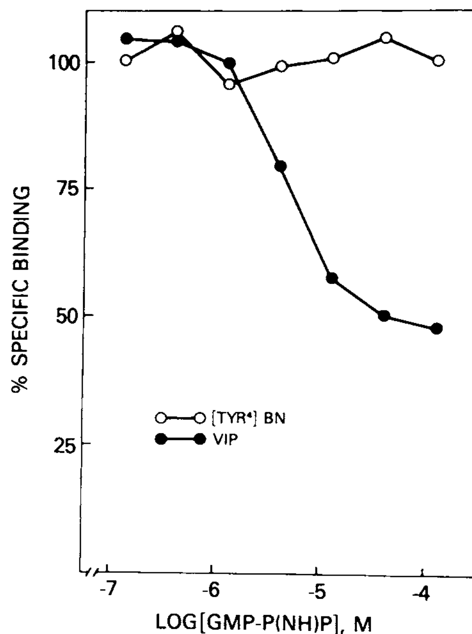

Fig. 1. Neuropeptide receptor binding as a function of nucleotide concentration. The amount of radiolabeled [Tyr^4]BN (○) and VIP (●) bound is determined as a function of GMP-P(NH)P concentration. Total and nonspecific binding determinations were made in triplicate. The mean amount of specific binding is indicated; the standard error was approximately 10% of the mean. All experiments were repeated at least three times.

RESULTS

The binding of [^{125}I-Try^4]BN and [^{125}I]VIP to crude rat brain homogenate was determined in the presence of varying concentrations of GMP-P(NH)P. Total binding of [^{125}I]VIP but not [^{125}I-Try^4]BN was decreased in a dose-dependent manner by micromolar concentrations of GMP-P(NH)P and GTP (data not shown). Nonspecific binding (binding in the presence of 1 M unlabeled peptide) was unaffected by guanine nucleotides (data not shown). Figure 1 shows that specific binding of [^{125}I]VIP but not [^{125}I-Tyr^4]BN was decreased in a dose-dependent manner. Specific VIP binding was reduced maximally by 60% using 20 μM GMP-P(NH)P. These data indicate that the guanine nucleotides GTP and GMP-P(NH)P inhibit high affinity binding of peptide to the VIP but not Try^4-BN receptor.

The ability of other nucleotides to inhibit [^{125}I]VIP binding was investigated. Table I shows that only GTP and GMP-P(NH)P inhibited binding at a 20 μM dose. GDP and GMP did not significantly inhibit binding, suggesting that a nucleotide triphosphate is required. Because neither ATP, CTP, ITP, or UTP had significant inhibitory effects, it appears that the guanine base is required. These data indicate that guanine nucleotide triphosphates may interact with a membrane bound component such that the binding of radiolabeled VIP to its receptor is impaired.

The effect of GMP-P(NH)P on the affinity of the receptor for radiolabeled VIP was investigated. The amount of [^{125}I]VIP bound to rat brain membranes was determined as a function of increasing concentration of unlabeled VIP in the presence and absence of 20

TABLE I. Ability of Various Nucleotides to Inhibit [^{125}I]VIP Binding

Nucleotide	% of control value ± SE
None	100
AMP	101 ± 7
ATP	106 ± 6
CMP	96 ± 5
CTP	95 ± 14
GMP	96 ± 7
GDP	90 ± 10
GTP	64 ± 12
GMP-P(NH)P	39 ± 7
ITP	92 ± 11
UMP	95 ± 1
UTP	92 ± 13

The mean value ± SE of four experiments is indicated. A nucleotide dose of 20 μM was used.

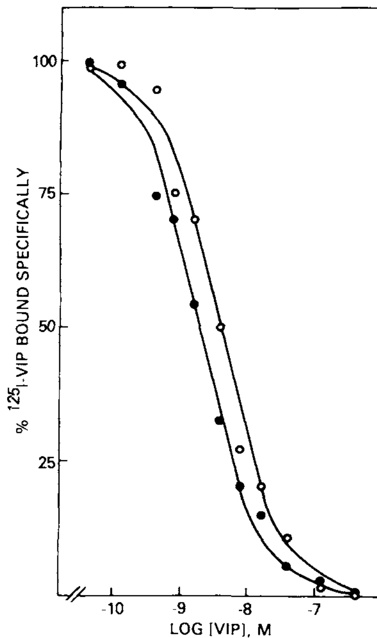

Fig. 2. Competitive inhibition of [^{125}I]VIP binding by unlabeled VIP in the presence (○) and absence (●) of 20 μM GMP-P(NH)P. The lines drawn represent the best fit assuming competitive inhibition when the dissociation constant is 2 (●) and 4 nM (○).

μM GMP-P(NH)P. Figure 2 shows that 10^{-10} M unlabeled peptide inhibits little specific binding, whereas 10^{-7} M VIP inhibits almost all specific binding. Binding is inhibited in a competitive manner, and the dose of unlabeled peptide required to inhibit 50% of the specific binding (IC_{50}) was 2 nM in the absence and 4 nM in the presence of GMP-P(NH)P. These data suggest that GMP-P(NH)P may reduce the amount of VIP bound at equilibrium through a reduction in affinity rather than in the number of VIP binding sites.

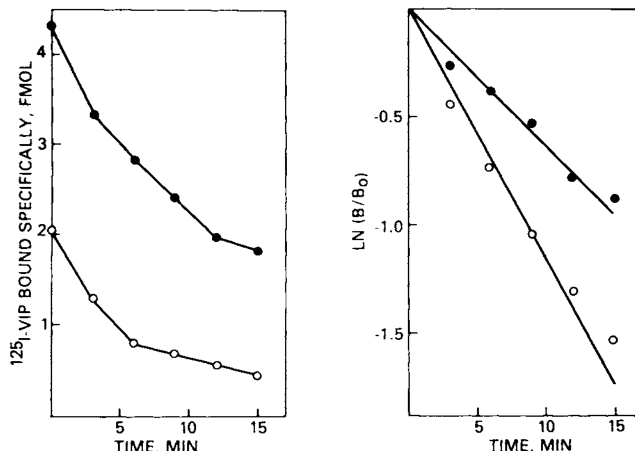

Fig. 3. Kinetics of [^{125}I]VIP dissociation from rat brain homogenate. The radiolabeled peptide was incubated with membranes to equilibrium (20 min) in the presence (○) and absence (●) of 20 μM GMP-P(NH)P. Then 1 μM unlabeled VIP was added. Left: The amount remaining bound is determined as a function of time. Right: Replot of the amount remaining bound (B) relative to the amount bound at equilibrium (B_0).

The effect of 20 μM GMP-P(NH)P on the kinetics of [^{125}I]VIP binding was investigated. Rat brain membranes bound radiolabeled VIP with the same association rate constant in the presence and absence of GMP-P(NH)P (data not shown). The rate of dissociation, however, was increased dramatically in the presence of GMP-P(NH)P (Fig. 3). The dissociation rate constant increased 80%; with $k_{-1} = 1.85 \times 10^{-3}$/sec in the presence of and $k_{-1} = 1.05 \times 10^{-3}$/sec in the absence of GMP-P(NH)P. These data show that in the presence of GMP-P(NH)P, the affinity of the CNS receptor for VIP is reduced as a consequence of an increase in the dissociation rate.

DISCUSSION

The guanine nucleotide triphosphates GTP and GMP-P(NH)P affect various hormone, neurotransmitter, and drug receptors coupled to adenylate cyclase. Because VIP stimulates adenylate cyclase activity in pancreatic acinar cells [3] and brain homogenates [6–8] whereas BN does not, we investigated the ability of nucleotides to inhibit VIP and [Tyr^4]BN brain receptor binding. The data presented here indicate that GTP and GMP-P(NH)P inhibit binding of [^{125}I]VIP but not [^{125}I-Try^4]BN. It is unlikely that this difference reflects the binding conditions used because preliminary data [T. Moody, unpublished] indicate that when both assays are conducted at 25°C in the presence of 2 mM $MgCl_2$, GMP-P(NH)P significantly inhibited the binding of [^{125}I]VIP but not [^{125}I-Tyr^4]BN. These data indicate that guanine nucleotide triphosphates affect central VIP receptors coupled to adenylate cyclase but not Tyr^4-BN receptors.

The ability of various nucleotides to inhibit [^{125}I]VIP binding was investigated. Table I shows that using a 20 μM dose, GMP-P(NH)P > GTP > GDP > GMP, whereas millimolar concentrations of any nucleotide inhibited up to 60% of the specific [^{125}I]VIP binding [T. Moody, unpublished]. This order of nucleotide potency mimics closely that of the β-adrenergic receptor [22], whereas for the opiate receptor GDP > GTP > GMP-P(NH)P > GMP

[15]. This difference may reflect the fact that opiate receptors, in contrast to β-adrenergic receptors, are coupled to adenylate cyclase in an inhibitory mode.

Receptor–VIP interactions are inhibited by GMP-P(NH)P as a result of a reduction in affinity. Figure 2 shows that 20 μM GMP-P(NH)P shifts the dose–response curve for unlabeled VIP to the right. The receptor's affinity for VIP is reduced approximately two-fold in comparison to the five-fold reduction in the β-adrenergic receptor for isoproterenol [23]. Also, preliminary analyses of Scatchard plot data [T. Moody, unpublished] indicate that 20 μM GMP-P(NH)P decreases the affinity of the receptor for VIP but does not affect the number of sites.

This reduction in affinity is reflected in an increased rate of dissociation of [^{125}I]VIP from its receptor. Rat brain membranes were incubated with [^{125}I]VIP in the presence and absence of 20 μM GMP-P(NH)P. Then 1 μM unlabeled VIP was added. The dissociation of radiolabeled peptide from the VIP receptor increased 80% in the presence of guanine nucleotide. In comparison, the association rate constant was not affected.

Previously, Robberecht et al [24] determined that when [^{125}I]VIP bound to the guinea pig brain receptor, GTP increased the rate of dissociation relative to VIP. This data is in accord with the 80% increase in the dissociation rate constant determined here. Similarly, they found that micromolar concentrations of guanine nucleotides decreased the amount of [^{125}I]VIP bound specifically at equilibrium by 50%, however, the order of nucleotide potency was GTP > GDP > GMP-P(NH)P > GMP. This difference in potency relative to that determined here may result from altered nucleotide stability; Robberecht et al [24] used guinea pig brain membranes and conducted their binding assay at 37°C.

Rodbell [21] postulates that guanine nucleotides such as GTP interact with a plasma membrane regulatory subunit, and that this interaction affects the coupling of agonist–receptor complex to adenylate cyclase as well as increases the rate of dissociation of agonist from receptor. Our observations suggest that GTP sensitivity is characteristic of those neuropeptide receptors which are coupled to adenylate cyclase.

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