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N-Methyl-D-Aspartate Receptor Antagonist Activity and Phencyclidine-Like Behavioral Effects of the Pentadecapeptide, [Ser^1]Histogranin

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SHUKLA, V. K., S. LEMAIRE, M. DUMONT AND Z. MERALI. *N-methyl-D-aspartate receptor antagonist activity and phencyclidine-like behavioral effects of the pentadecapeptide, [Ser^1]histogranin.* PHARMACOL BIOCHEM BEHAV **50**(1) 49–54, 1995.—The behavioral and pharmacologic profiles of [Ser^1]histogranin ([Ser^1]HN) were assessed by monitoring its ability to displace the binding of the specific *N*-methyl-D-aspartate (NMDA) receptor ligand, [^{3}H]CGP 39653, to block the convulsant effects of NMDA and other excitatory agents in mice, and to produce phencyclidine (PCP)-like behavioral effects in rats. The peptide potently inhibited [^{3}H]CGP 39653 binding to membrane preparations of rat brain with an IC_{50} of 198 nM and a maximal inhibition of 34% of the specific binding activity. Saturation binding experiments with [^{3}H]CGP 39653 in the absence and presence of [Ser^1]HN (2 μM) indicated that the inhibitory effect of the peptide was noncompetititive, producing a decrease in the maximal number of binding sites (B_{max} of 62.5 fmol/mg protein as compared with 91.3 fmol/mg protein in control), but no significant change in the affinity (K_d of 4.5 nM as compared with 5.1 nM in control). Intracerebroventricular (ICV) injection of [Ser^1]HN (10–100 nmol) in mice evoked a dose-dependent and selective blockade of NMDA-induced convulsions. In rats, [Ser^1]HN (2.5–100 nmol, ICV) produced dose-dependent stereotypy, ataxia, and locomotion similar to those observed with PCP, at doses ranging between 50 and 400 nmol. The data indicate that [Ser^1]HN noncompetitively interacts with the NMDA receptor, an action that goes along with its in vivo NMDA receptor antagonist activity and PCP-like behavioral effects.

Histogranin *N*-Methyl-D-aspartate Phencyclidine Convulsion Stereotypy Ataxia Locomotion

THE PROTOTYPIC excitatory amino acids, glutamate and aspartate, which are abundantly present in the mammalian central nervous system, have been recognized as important metabolic and neurotransmitter candidates with neurotoxic potential. Synaptic receptors for these putative excitatory amino acid neurotransmitters are currently classified into several distinct subgroups according to their preferred agonists, including the ionotropic receptors for *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionate, and kainic acid (16,25). Stimulation of the NMDA receptor has been involved in physiologic and pathophysiologic processes that include learning and memory processes and neurodegenerative insults such as cerebral ischaemia (3,16).

Various naturally occurring substances have been suggested as endogenous modulators of glutamate and/or aspartate-induced excitations including kynurenic acid (4,5,18), and glutathione (6). Histogranin (HN; H-Met-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH), a pentadecapeptide isolated from the adrenal medulla, was previously shown to possess NMDA receptor antagonist activity (13). This peptide is present in secretory granules and is released from perfused adrenal glands upon cholinergic receptor stimulation (10,13). The peptide possesses specific binding sites in membrane preparations of rat brain (20) and human peripheral blood lymphocytes (11). The binding of [^{125}I][Ser^1]HN to brain membranes is not affected by the presence

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of specific ligands of phencyclidine (PCP), sigma (σ), dopamine, nicotine, and muscarine receptors (20), but the binding of the competitive NMDA receptor antagonist, [^{3}H]CGP 39593, is inhibited by the presence of synthetic HN (13). Support for the specific interaction of HN with the NMDA receptor is also provided by its specific blockade of NMDA-induced convulsions in mice (13). The aim of the present investigation was to better define the type of interaction of HN with the NMDA receptor using a chemically stable analog of the compound, [Ser^1]HN (20). In addition, in vivo NMDA receptor antagonist activity and PCP-like behavioral effects were also assessed.

METHODS

Drugs

N-Methyl-D-aspartate and kainic acid were purchased from Sigma Chemical Co. (St. Louis, MO). α-Amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), (+)bicuculline, and pentylenetetrazole were products of Research Biochemical Incorporated (Natick, MA). (±)3-(2-carboxypiperazine-4-yl)-propyl-1-propionic acid (CPP) was obtained from Tocris Neuramin (Essex, UK). [^{3}H]2-amino-4-propyl-5-phosphono-3-pentanoic acid ([^{3}H]CGP 39653; 50 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Ser^{1}HN was synthesized in our laboratory by the solid-phase procedure (15) as described previously (12).

[^{3}H]CGP 39653 Binding

[^{3}H]CGP 39653, a specific NMDA receptor ligand, was used to monitor the interaction of Ser^{1}HN with NMDA receptors in membrane preparations of rat brain (21). Typical binding assays were performed in 5 mM Tris-HCl buffer (pH 7.4) (buffer A) at 4°C for 60 min with a 2-ml aliquot of the membrane preparation (0.4 mg protein/ml) in the presence of [^{3}H]CGP 39653 (5 nM) and various concentrations of Ser^{1}HN, as indicated. Incubations were terminated by filtration under reduced pressure through GF934AH Whatman filters (Springfield Mill, Maidstone, Kent, England) pretreated with 0.05% polyethylenimine. Filters were washed with 4 × 3 ml aliquots of ice-cold buffer A, placed in liquid scintillation vials along with 10 ml Ecolume (ICN Biochemical, Costa Mesa, CA) and counted in a Beckman liquid scintillation counter (Beckman Scientific Instrument Div., Irvine, CA). Nonspecific binding of [^{3}H]CGP 39653 was determined in the presence of 10 μM CPP. Specific binding was defined as the difference between the radiolabel bound in the presence and absence of CPP. All the experiments were repeated three times in duplicate; values represent the mean ± SE. The concentration that produced 50% inhibition of [^{3}H]CGP 39653 binding (IC_{50}) was derived using the nonlinear least-square computer fitting program CDATA (EMF Software, Knoxville, TN). Saturation experiments were performed in the absence (control) or presence of Ser^{1}HN (2 μM) with increasing concentrations of [^{3}H]CGP 39653 between 0.5 and 20 nM. The equilibrium dissociation constant (K_d) and the maximum binding capacity (B_{max}) were obtained from Scatchard plot analysis using the iterative curve fitting program BDATA (EMF Software).

Anticonvulsive Activity in Mice

Male Swiss Webster mice ((SW)fBR; Canadian Breeding Farm, St-Constant, Quebec, Canada) weighing 25–30 g were housed five per cage in a room with controlled temperature (22 ± 2°C), humidity, and artificial light (0630–1900 h). The animals had free access to food and water and were used after a minimum of 4 days of acclimation to the housing conditions. Freehand intracerebroventricular (ICV) injections into the lateral ventricles of the conscious mouse were made as described previously (1), using the method of Clark et al. (2). The injections were made using a no. 27 gauge, 0.25-in needle attached to a 500-μl Hamilton syringe and an automatic dispenser (PB 600; Hamilton Co., Reno, NV). The needle was filled with polyethylene tubing, leaving 3 mm of the needle tip exposed. The point of injection was on an imaginary line drawn through the anterior lobe of the ears and from an imaginary midsagital line. The whole injection procedure was completed within 10–15 s, so that the animal should suffer minimal discomfort and pain. The convulsant drugs NMDA (0.25–1 nmol), AMPA (0.25–2.0 nmol), kainic acid (0.25–0.75 nmol), bicuculline (1–10 nmol), or pentylenetetrazole (1.25–5.00 μmol) were injected ICV in 10 μl vol. After injection, the animals were individually housed in transparent polycarbonate cages (29 × 19 × 13 cm) and observed for a minimum of 30 min for the signs of convulsions. The following responses were noted during the observation period: a) mild myoclonus (moderate jerky movement of one or two limbs); b) whole-body clonus (dramatic and violent movements involving all the limbs and the body leading to loss of righting reflex); c) clonic-tonic seizures consisting of the following successive components: wild running characterized by episodes of running with explosive jumps, clonus, and, finally, tonus characterized by extreme rigidity of the whole body. Groups of 15 animals were preinjected with saline (control) or the indicated dose of the peptide before the injection of the indicated dose of the tested analeptic. The animals were scored as showing seizure activity when one or more of the three responses mentioned earlier were present, and in each group the number of animals showing these behavioral signs of convulsions was recorded. The protective effect of Ser^{1}HN (5–100 nmol/10 μl/mouse, ICV) was verified by injection of the peptide 5 min before the administration of convulsive agents. Quantal dose–response data (CD^{50}, relative potency, 95% confidence limits) were obtained by the method of Litchfield and Wilcoxon using the computer program of Tallarida and Murray (23).

Behavioral Activity in Rats

All experiments were conducted with male Sprague–Dawley CD rats (300–400 g) obtained from Charles River Laboratories (Rochefort, Quebec, Canada), housed individually and maintained on a 12-h light–dark cycle with the lights on at 0600 h. The room temperature was maintained at 21–23°C with 60% relative humidity. All test sessions occurred during the light cycle, between 0900 and 1130 h. Rats (n = 16) anesthetized with sodium pentobarbital (Somnatol) were stereotaxically implanted with permanent guide cannula (Plastics One, Roanoke, VA) aimed at the third ventricle (coordinates from Bregma with level skull: A-P 4.3 mm, Lat 0.0 mm, and D-V 4.3 mm). After 7 days of recovery, one group of rats (n = 8) was administered vehicle (saline) or [Ser1HN (2.5, 25, 50, and 100 nmol), according to Latin-square design. Treatments were spaced 3 days apart. Ser^{1}HN doses were administered into the third ventricle, delivered in a 4-μl vol over 60 s, via an injection cannula (0.5 mm longer than the guide cannula). Data were analyzed using repeated measures analysis of variance (ANOVA) followed by post-hoc Tukey's tests. Locomotor activity was defined as the distance transversed by rats (in cm) over 1 h following injection (7). Stereotyped behavior was defined as isolated motor acts or partial sequences of more

complex behavior. Stereotypy was manifested by intense sniffing, back pedaling, continuous turning, and grooming. It was scaled according to the methods described previously (22). Ataxia was defined as impairment in the ability of the animal to execute coordinated motor responses leading in the extreme to incapacitation. It was mostly manifested by awkward movements or loss of balance. The scale items for assessing Ser[1]HN-induced ataxia were the same as those described by Sturgeon et al. (22). This experiment was repeated in a separate group of rats (n = 8) that received PCP (50, 100, 200, and 400 nmol, ICV) in place of Ser[1]HN.

RESULTS

Effect of Ser[1]HN on the Binding of [^{3}H]CGP 39653

Ser[1]HN was tested for its possible modulatory effect on the binding of specific σ ([^{3}H][+]pentazocine), PCP ([^{3}H]MK-801), and NMDA ([^{3}H]CGP 39653) receptor ligands to rat brain membrane preparations. Ser[1]HN (10^{-9}–10^{-4} M) dose-dependently displaced the specific binding activity of [^{3}H]CGP 39653 (Fig. 1) but had no effect on the binding of (+)[^{3}H]pentazocine and [^{3}H]MK-801 (5 nM, not shown). Analysis of the competition curve with [^{3}H]CGP 39653 binding revealed that the presence of the peptide maximally inhibited 34% of the binding sites with an IC_{50} of 198 nM as compared with 551 nM for L-glutamate (Fig. 1). Equilibrium saturation experiments were also performed at 4°C for 60 min with increasing concentration of [^{3}H]CGP 39653 (5–20 nM) in the absence or presence of Ser[1]HN (2 μM) (Fig. 2). Scatchard plot analysis indicated that the peptide significantly decreased the maximal binding activity (B_{max} from 91.3 ± 6.3 to 62.5 ± 5.6 fmol/mg protein), but failed to affect significantly the K_d (4.5 ± 0.5 nM as compared with 5.1 ± 0.6 nM, control).

Anticonvulsive Activity of Ser[1]HN

Intracerebroventicular injection of Ser[1]HN in mice produced a brief increase in locomotion in some animals, but when injected 5 min before NMDA (0.5, 0.75, and 1.00 nmol, ICV), it dose-dependently blocked NMDA-induced convulsions (Fig. 3). The dose of NMDA required to produce convulsions in 50% of the animals (CD_{50}) was increased in the presence of 10, 50, and 100 nmol of Ser[1]HN from 0.62 nmol/mouse to 0.80, 0.99, and 1.29 nmol/mouse, respectively. This is illustrated by the significant decrease in potency ratios as compared with NMDA alone (Fig. 3, Table 1). The slopes of the quantal NMDA dose response curves after the administration of 0, 10, 50, and 100 nmol of Ser[1]HN were 2.82 ± 0.26, 3.38 ± 0.33, 3.42 ± 0.33, 2.68 ± 0.28, and 2.14 ± 0.22, respectively. They did not differ significantly from one another. On the other hand, AMPA-, kainic acid-, bicuculline-, and pentylenetetrazole-induced convulsions were not significantly affected by the presence of the peptide (no significant change in potency ratios; Table 1).

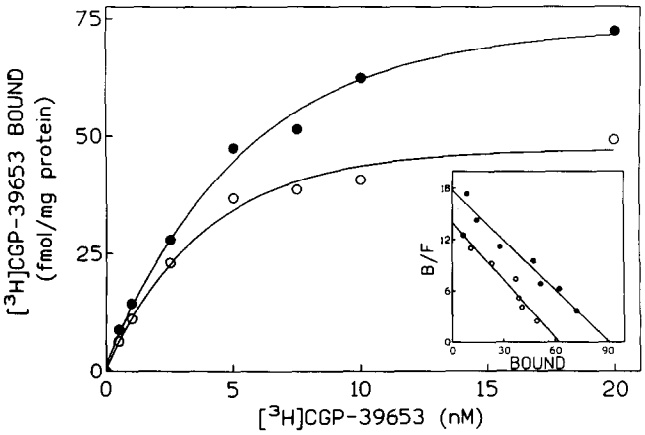

FIG. 2. Saturation curves and scatchard plots (inset) of specific [^{3}H]CGP39653 binding to rat brain membrane preparations in absence (●) and presence (○) of [Ser[1]]histogranin. Binding experiments were performed as described under METHODS, and each value represents the mean of three experiments conducted in duplicate.

Behavioral Effects of Ser[1]HN in Rats

Ser[1]HN injected ICV in rats produced dose-related stereotypy, ataxia, and locomotion (Table 2). Stereotypy and ataxia

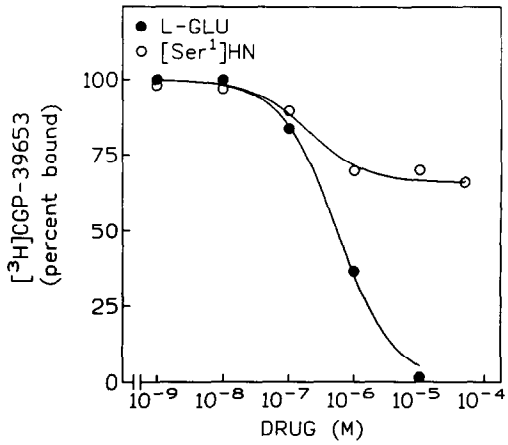

FIG. 1. Modulatory effects of increasing concentrations of [Ser[1]]histogranin ([Ser[1]]HN) (●) and L-Glu (○) on the binding of the *N*-methyl-D-aspartate receptor ligand, [^{3}H]CGP39653 (5nM) to membrane preparations of rat brain. The curves represent the mean of three replicate experiments.

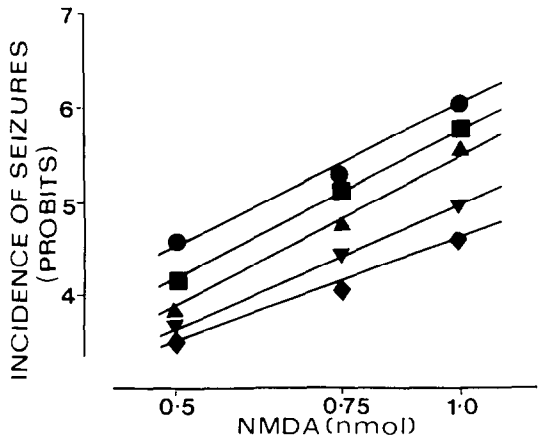

FIG. 3. Probit-log dosage regression curves for convulsions induced by *N*-methyl-D-aspartate (NMDA) in mice in the absence (●) and presence of different doses (5, ■; 10, ▲; 50, ▼; and 100,◆nmol/mouse) of [Ser[1]]histogranin.

TABLE 1

EFFECT OF [SER^1]HISTOGRANIN ([SER^1]HN) ON *N*-METHYL-D-ASPARTATE (NMDA)-, α-AMINO-3-HYDROXY-5-METHYLISOXAZOLE-4-PROPIONATE (AMPA)-, KAINIC ACID, BICUCULLINE, AND PENTYLENETETRAZOL-INDUCED CONVULSIONS IN MICE (ICV)

Convulsant	[Ser^1]HN (nmol/Mouse)	CD_{50} (nmol/Mouse) 95% Confidence Limit	Potency Ratio (95% Confidence Limit)
NMDA	0 (Saline)	0.61 (0.52–0.71)	1
	5	0.70 (0.59–0.82)	0.87 (0.70–1.09)
	10	0.80 (0.65–0.98)	0.76 (0.59–0.98)*
	50	0.99 (0.72–1.38)	0.62 (0.44–0.88)*
	100	1.29 (0.88–1.89)	0.48 (0.32–0.72)*
AMPA	0 (Saline)	0.33 (0.18–0.59)	1
	10	0.35 (0.27–0.44)	0.94 (0.50–1.78)
	50	0.38 (0.26–0.58)	0.85 (0.42–1.74)
Kainic acid	0 (Saline)	0.40 (0.31–0.50)	1
	10	0.43 (0.32–0.57)	0.93 (0.64–1.34)
	50	0.35 (0.24–0.51)	1.13 (0.73–1.76)
Bicuculline	0 (Saline)	2.68 (1.41–5.11)	1
	10	3.69 (1.95–6.99)	0.73 (0.29–1.80)
	50	3.14 (1.52–6.50)	0.85 (0.32–2.25)
Pentylenetetrazol	0 (Saline)	2367 (1513–3702)	1
	10	2741 (1639–4582)	0.86 (0.44–1.71)
	50	2617 (1634–4191)	0.90 (0.47–1.73)

*$p < 0.05$.

were observed at a lower dose (25 nmol) than locomotion (100 nmol). These effects were significantly increased with the dose. Similarly, PCP produced dose-dependent stereotypy, ataxia, and locomotion, but at higher doses (50–400 nM). Again, stereotypy and ataxia were observed at a lower dose (50 nmol) than locomotion (100 nmol).

DISCUSSION

Histogranin was first isolated from bovine adrenal medulla, a tissue recognized as an important source of neuropeptides (9). Ser^{1}HN, a chemically stable analog of HN, was previously synthesized and used to characterize HN binding sites in membrane preparations of rat brain. [^{125}I]Ser^{1}HN binds to sites that display the characteristics of receptors, namely saturability, reversibility, specificity, and proteolytic enzyme sensitivity (20). The present study indicates that Ser^{1}HN is a potent inhibitor of the binding of [^{3}H]CGP 39653, a selective NMDA receptor ligand. Thirty-four percent of the specific binding sites is sensitive to the presence of the peptide, and the interaction between the peptide and the radioactive ligand is most likely noncompetitive, with the B_{max} being decreased from 91.3 to 62.5 pmol/mg protein and the K_d remaining the same. The NMDA receptor complex is linked to an Na^+/Ca^{2+} ion channel with a high calcium conductance (14). The opening of the channel consecutive to stimulation of the

TABLE 2

BEHAVIORAL EFFECTS OF CENTRALLY ADMINISTERED [Ser^1]HISTOGRANIN ([Ser^1]HN) AND PHECYCLIDINE (PCP) IN THE RAT

Treatment	Dose (nmole)	Stereotypy	Ataxia	Locomotion
Control	0 (Saline)	0.5 ± 0.1	0.15 ± 0.1	324 ± 51
[Ser^1]HN	2.5	0.6 ± 0.15	0.13 ± .12	250 ± 134
	25	1.5 ± 0.21*	3.25 ± 0.86*	310 ± 103
	50	1.6 ± 0.22*	5.0 ± 0.13*	246 ± 90
	100	2.6 ± 0.51†	4.5 ± 0.5*	1287 ± 277†
PCP	50	1.6 ± 0.2*	0.62 ± 0.6*	435 ± 95
	100	1.8 ± 0.16*	0.86 + 0.7*	858 + 241*
	200	1.67 ± 0.21*	2.1 ± 1.0*	634 ± 173*
	400	1.83 ± 0.16*	0.83 ± 0.83*	2351 ± 273†

*$p \leq 0.05$.
†$p \leq 0.01$.

NMDA receptor is controlled by distinct binding domains on the receptor complex, including sites for NMDA, PCP, Mg^{2+}, Zn^{2+}, and polyamines (24). Olney (17) hypothesized that the normal function of the NMDA receptor complex depends on the dynamic equilibrium among multiple facilitatory and inhibitory factors. A noncompetitive nature of the inhibition of [^{3}H]CGP 39653 binding by Ser^{1}HN would suggest that the interaction of the peptide with its specific binding site may cause an allosteric modulation in the conformation of the NMDA binding site leading to blockade of NMDA receptor-mediated activity.

Many compounds that antagonize mammalian excitatory amino acid receptors have anticonvulsant and neuroprotective effects (19). The possibility that Ser^{1}HN modulates the stimulation of NMDA receptors was verified in mice. Ser^{1}HN (10–100 nmol/mouse ICV) produced dose-dependent blockade of NMDA-induced convulsions, but did not affect the convulsions induced by AMPA, kainic acid, bicuculline, and pentylenetetrazole. The threshold effective dose (10 nmol) for Ser^{1}HN against NMDA-induced convulsions was the same as that reported for HN (13). Such potency can be favorably compared with that of the noncompetitive NMDA antagonist, PCP (8). The determination of the mode of action of HN and related peptides in regard to their NMDA antagonist activity will necessitate further studies.

The behavioral effects of PCP and Ser^{1}HN were qualitatively similar. However, quantitatively, there were differences between the two agents. At lower doses (25–100 nmol) both Ser^{1}HN and PCP induced stereotypy and ataxia; Ser^{1}HN was more potent and efficacious in eliciting these effects. At higher doses (>100 nmol), both compounds stimulated locomotor activity; however, more extensive dose-response studies would be needed to evaluate their efficacy. The behavioral effects of Ser^{1}HN were dose-dependent and probably were not caused by a direct interaction of the peptide with the PCP or NMDA receptors, because the binding of [^{125}I]Ser^{1}HN in rat brain membranes was not affected by PCP and NMDA receptor ligands (20). However, an allosteric modulation by Ser^{1}HN of some domain linked to the NMDA receptor complex may result in a behavioral profile that resembles that of PCP, with PCP itself considered to be a noncompetitive inhibitor of the NMDA receptor and a potent neuroprotective agent (19,24).

In summary, the present study demonstrates that Ser^{1}HN is a potent inhibitor of the binding of the NMDA receptor ligand, [^{3}H]CGP 39653. The inhibition reaches a maximum of 34% of specific [^{3}H]CGP 39653 binding, suggesting that HN interacts with a specific subtype of NMDA receptors. This interaction is most likely noncompetitive, implying that the peptide possesses its own binding site distinct from the NMDA receptor (20). Finally, the selective blockade of NMDA-induced convulsions in mice, and the PCP-like behavioral effects of Ser^{1}HN, further support the hypothesis that HN may be considered an endogenous modulator of the NMDA receptor.

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