ELSEVIER

European Journal of Pharmacology 283 (1995) 251–254

ejp

Short communication

# The pentadecapeptide [Ser$^1$]histogranin impairs passive avoidance learning in mice

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Received 10 April 1995; revised 3 July 1995; accepted 7 July 1995

**Abstract**

The peptides, histogranin and [Ser$^1$]histogranin, were recently shown to modulate NMDA receptor function. In the present study, the effects of intracerebroventricular (i.c.v.) administration of [Ser$^1$]histogranin and of the histogranin receptor antagonist, histogranin-(1–10), were examined on step-down type passive avoidance learning in mice. [Ser$^1$]Histogranin (30–60 nmol) impaired retention, after post-training administration, but not when it was administered just prior to the retention assay. Histogranin-(1–10) (60 nmol) facilitated learning during training, without affecting retention. Co-administration of histogranin-(1–10) with [Ser$^1$]histogranin (60 nmol each) led to a significant prevention of [Ser$^1$]histogranin-induced learning impairment. These results indicate that [Ser$^1$]histogranin impairs passive avoidance learning according to the pattern of NMDA receptor antagonists and involving specific histogranin sites.

*Keywords:* Histogranin; NMDA receptor; Passive avoidance; Learning; Memory; (Mouse)

## 1. Introduction

Histogranin (H-Met-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH) is a pentadecapeptide originally isolated from the adrenal medulla, and named after its structural homology with fragment-(86–100) of histone H4 (Lemaire et al., 1993, 1995). The peptide is present in high concentration in chromaffin granules and is released from perfused bovine adrenal glands, suggesting a neuropeptide function (Lemaire et al., 1993). [$^{125}$I][Ser$^1$]Histogranin possesses sites in rat brain membranes that display characteristics of a specific receptor namely high affinity, trypsin sensitivity, specificity, saturability and reversibility (Rogers and Lemaire, 1993). In addition, synthetic histogranin non-competitively inhibits the binding of [$^3$H]CGP 39653, a competitive antagonist of the NMDA receptor. In vivo experiments indicate that intracerebroventricular (i.c.v.) administration of histogranin to mice specifically blocks convulsions induced by *N*-methyl-D-aspartate (NMDA) without affecting those induced by (*R*,*S*)-α-amino-3-hydroxy-5-methyl-14-isoxazole propionate (AMPA), kainate and bicuculline (Lemaire et al., 1993). The chemically stable analog, [Ser$^1$]histogranin (10–100 nmol i.c.v. in mice), displays the same potency as the parent peptide to antagonize NMDA-induced convulsions (Shukla et al., 1995). Both binding and anticonvulsant activities of histogranin and related peptides require the full-length 15 amino acid peptide (Rogers and Lemaire, 1993), with the exception of histogranin-(1–10) which possesses a high binding affinity for the histogranin receptor and acts as an antagonist towards the anti-NMDA activity of histogranin (Prasad et al., 1995). In rats, [Ser$^1$]histogranin (100 nmol i.c.v.) produces behavioural effects (stereotypy, ataxia and hyperlocomotion) that resemble those observed with the non-competitive NMDA receptor antagonist, phencyclidine (Shukla et al., 1995).

The activation of the NMDA receptor complex plays a prominent role in learning and memory processes. The first evidence for the involment of the NMDA

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*SSDI* 0014-2999(95)00413-0

receptor in cognitive processes was reported by Morris et al. (1986). The competitive NMDA receptor antagonist, aminophosphonovaleric acid (AP5), impaired spatial learning in a rat water maze assay. In hippocampal CA1 pyramidal neurons, AP5 blocked the initiation of long-term potentiation, a proposed substrate of learning and memory (Morris et al., 1986). Competitive (AP5) and non-competitive (phencyclidine, dizocilpine) NMDA receptor antagonists also blocked working memory in the radial maze and impaired acquisition of the passive avoidance response in rats (Danysz et al., 1988; DeNoble et al., 1990; Venable and Kelly, 1990). Similar observations were made in mice, particularly regarding passive avoidance response and alternation behaviour in the Y maze (Parada-Turska and Turski, 1990; Maurice et al., 1994a). The aim of the present study was to examine the effects of [Ser$^1$]histogranin on acquisition and retrieval of the passive avoidance response in mice. The histogranin receptor antagonist, histogranin-(1–10), was also investigated, alone and in combination with [Ser$^1$]histogranin.

## 2. Material and methods

### 2.1. Animals

Male Swiss mice (Iffa-Credo, L'Arbresle, France), aged 5–6 weeks and weighing 33–40 g, were used. They were housed in plastic cages, with free access to standard laboratory food and water, and kept in a controlled environment (23 ± 1°C, 50% humidity), under a 12-h light/dark cycle (light on at 7:00 a.m.). The mice were used for the experiments after they were adapted to laboratory conditions for at least 3 days. Experiments were carried out between 10:00 a.m. and 6:00 p.m. in an air-regulated and soundproof laboratory (23 ± 1°C, 40% humidity), where the mice were allowed to settle down for 30 min before each experiment.

### 2.2. Peptide preparations and administration procedures

[Ser$^1$]Histogranin and histogranin-(1–10) were synthesized in the laboratory of one of us (S.L.) by the solid-phase procedure as described previously (Prasad et al., 1995). Peptides were dissolved in double-distilled sterile water (vehicle). The i.c.v. administration of peptides was performed as described by Haley and McCormick (1957) and by us (Maurice et al., 1994b). Peptides or vehicle (3 μl) were delivered gradually within approximately 3 s; the mice behaving normally within 1 min after injection. The administration site was checked by injecting Indian ink in preliminary experiments.

### 2.3. Step-down passive avoidance test in mice

The apparatus consisted of a transparent acrylic cage (30 × 30 × 40 cm high) with a grid floor, inserted in a semi-soundproof outer box (35 × 35 × 90 cm high). The cage was illuminated with a 15 W lamp during the experimental period. A wooden platform (4 × 4 × 4 cm) was fixed in the centre of the grid floor. Electric shocks (1 Hz, 500 ms, 40 V DC) were delivered to the grid with an isolated pulse stimulator (Model 2100, A-M Systems, Everett, WA, USA). Training was carried out in two similar sessions. Each mouse was placed on the platform in the center of the cage. When the mouse stepped down and placed all its paws on the grid floor, shocks were delivered for 15 s. Step-down latency, and the number of flinching reactions and vocalizations were measured. Animals showing a latency in the criterion range (3–30 s) during the first training session were used for the second session and the retention test. More than 95% of the animals met

Table 1
Effect of [Ser$^1$]histogranin ([Ser$^1$]HN) and histogranin-(1–10) (HN-(1–10)) on step-down passive avoidance training in mice

| Peptide (nmol i.c.v.) | | $n$ | First training session | | | Second training session | | |
|---|---|---|---|---|---|---|---|---|
| | | | SDL (s) Median [I.R.] | Vocalizations Mean ± S.E.M. | Flinching actions Mean ± S.E.M. | SDL (s) Median [I.R.] | Vocalizations Mean ± S.E.M. | Flinching actions Mean ± S.E.M. |
| Vehicle | | 33 | 6 [3–11] | 1 ± 0.3 | 11 ± 0.6 | 14 [6–45] [a] | 0 ± 0.1 | 7 ± 0.7 [a] |
| [Ser$^1$]HN | (30) | 26 | 10 [5–19] | 1 ± 0.6 | 10 ± 0.8 | 20 [8–43] [a] | 1 ± 0.4 | 9 ± 0.8 |
| | (60) | 28 | 7 [3–16] | 0 ± 0.2 | 9 ± 0.5 | 6 [3–14] [b] | 0 ± 0.1 | 8 ± 0.6 |
| HN-(1–10) | (60) | 17 | 10 [7–24] | 1 ± 0.4 | 10 ± 0.7 | 60 [10–60] [a,c] | 0 ± 0.0 | 9 ± 0.8 |
| [Ser$^1$]HN | (60) + | | | | | | | |
| HN-(1–10) | (60) | 27 | 10 [5–17] | 1 ± 0.4 | 10 ± 0.7 | 12 [6–27] | 1 ± 0.3 | 9 ± 0.6 |
| Kruskal-Wallis ANOVA | | | $H = 9.61$ | $H = 1.60$ | $H = 2.57$ | $H = 22.59$ | $H = 7.05$ | $H = 4.66$ |
| | | | $P < 0.05$ | $P > 0.05$ | $P > 0.05$ | $P < 0.01$ | $P > 0.05$ | $P > 0.05$ |

[a] $P < 0.01$ vs. the same treatment group in the first training session (Wilcoxon's paired non-parametric test). [b] $P < 0.05$, [c] $P < 0.01$ vs. the vehicle-treated group in the same training session (Dunn's non-parametric test).

this criterion. The second session was carried out 90 min after the first. Animals staying up to 60 s on the platform were considered as remembering the task and were removed from the platform without being given further electric shocks. The retention test was carried out 24 h after training, in a similar manner, except that the electric shocks were not applied to the grid floor. Thus, each mouse was placed on the platform, and the latency was recorded with a maximum cut-off time of 300 s.

### 2.4. Data analysis

The results are expressed as the means ± S.E.M., except for latencies, expressed in terms of medians and interquartile ranges. The data did not show a normal distribution, since maximum cut-off times had been set (non-parametric data). They were analyzed by the Kruskal-Wallis analysis of variances by ranks, post-hoc comparisons being made with the Dunn's non-parametric multiple comparisons test. Data from the same treatment group between the two training sessions in the step-down passive avoidance test in mice were analyzed with the Wilcoxon's paired non-parametric test. The criterion for statistical significance was $P < 0.05$.

## 3. Results

[Ser$^1$]Histogranin and histogranin-(1–10), alone and in combination, were administered 10 min after the first training session, the second training session being performed 90 min after the first one. The behaviours observed during the two training sessions are summarized in Table 1. During the second training session, the vehicle-treated group exhibited a significant increase in latencies ($P < 0.01$, Wilcoxon's test), with decreases in the numbers of vocalizations ($P > 0.05$) and of flinching reactions ($P < 0.01$), indicating that the animals already learned the task and showed some habituation to the footshocks. A significant increase in latencies between the two training sessions was also observed for the [Ser$^1$]histogranin-treated group, at 30 nmol ($P < 0.01$), but not at 60 nmol ($P > 0.05$ and $P < 0.05$ vs. the vehicle-treated group). A significant increase in latencies was also observed for the histogranin-(1–10)-treated group ($P < 0.01$), the increase being significantly higher than that observed for the vehicle-treated group ($P < 0.01$; Table 1). No modification in latencies was observed for the group treated with the combination of [Ser$^1$]histogranin and histogranin-(1–10) at 60 nmol each. Furthermore, no significant modification in the sensitivity to the footshocks, in terms of vocalizations and flinching actions, was observed in the peptide-treated groups.

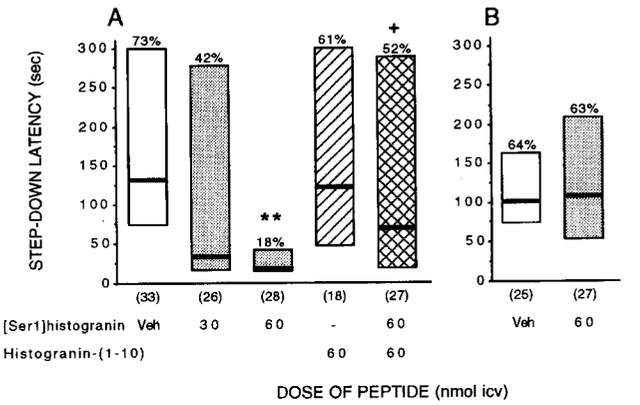


Fig. 1. Effect of [Ser$^1$]histogranin and histogranin-(1–10) during retention in the step-down passive avoidance test in mice. (A) Peptides were administered 10 min after the first training session. (B) The peptide was administered 30 min before retention. Columns represent the median latency of the number of animals indicated within each column. Interquartile range are indicated between brackets. Veh: vehicle. $^{**}P < 0.01$ vs. the vehicle-treated group; $^{+}P < 0.05$ vs. the [Ser$^1$]histogranin (60 nmol)-treated group; Dunn's test.

The latencies measured during the retention test are presented in Fig. 1A. The [Ser$^1$]histogranin-treated groups showed a dose-dependent decrease in latencies, significance being achieved for the group treated with 60 nmol ($P < 0.01$; Dunn's test; Fig. 1A). The histogranin-(1–10) treatment did not affect latencies, and the co-administration of [Ser$^1$]histogranin and histogranin-(1–10) (60 nmol each) led to a significant prevention of the [Ser$^1$]histogranin-induced decrease in latencies ($P < 0.05$ vs. the [Ser$^1$]histogranin-treated group; Fig. 1A).

In a second set of experiments, [Ser$^1$]histogranin (60 nmol) was administered 30 min before the retention assay (Fig. 1B). Under these conditions, the administration of the peptide did not evoke any significant change in latencies.

## 4. Discussion

The present results show that central administration of histogranin-related peptides affects in a significant manner the mnemonic processes involved in passive avoidance conditioning in mice. The histogranin analog, [Ser$^1$]histogranin, dose-dependently decreased passive avoidance acquisition. Several lines of evidence support the concept that the learning impairment evoked by [Ser$^1$]histogranin may be mediated by the NMDA receptor antagonist activity of the peptide. Firstly, the learning impairment now observed with [Ser$^1$]histogranin depends upon the time when the peptide is administered during the learning process. No effect was observed on retrieval, i.e., when the peptide was administered immediately before the re-

tention assay, whereas significant learning impairment was observed when the peptide was administered between the two training sessions. Similar profiles of amnesic activity were reported for competitive and non-competitive NMDA receptor antagonists (Parada-Turska and Turski, 1990; DeNoble et al., 1990; Venable and Kelly, 1990). In addition, previous studies indicate that both histogranin and [Ser$^1$]histogranin are potent non-competitive inhibitors of [$^3$H]CGP 39653 binding to the NMDA receptor (Lemaire et al., 1993; Shukla et al., 1995), although the binding of [$^{125}$I][Ser$^1$]histogranin to rat brain membranes is not affected itself by specific ligands of the NMDA, phencyclidine, sigma, dopamine, nicotine and muscarine receptors (Rogers and Lemaire, 1993). In vivo, both histogranin and [Ser$^1$]histogranin show the same potency (dose range, 10–100 nmol i.c.v. in mice) to antagonize NMDA-induced convulsions (Lemaire et al., 1993; Shukla et al., 1995) while slightly higher doses (100 nmol i.c.v. in rats) of [Ser$^1$]histogranin induce a behavioural profile (stereotypy, ataxia and hyperlocomotion) that resembles that of the non-competitive NMDA receptor antagonist, phencyclidine (Shukla et al., 1995).

A major point in this study is the antagonist activity of histogranin-(1–10) towards the learning impairment induced by [Ser$^1$]histogranin. Alone, histogranin-(1–10) did not affect retention, but administered in combination with [Ser$^1$]histogranin, it significantly prevented the amnesic effect of [Ser$^1$]histogranin. These data are consistent with the antagonism by histogranin-(1–10) of the anticonvulsive activity of histogranin (Prasad et al., 1995) and indicate that specific histogranin receptors are most likely involved in the amnesic effects of [Ser$^1$]histogranin. The facilitation of the learning process by histogranin-(1–10), an effect that was assessed from the increased latency values in the second training session as compared with vehicle-treated animals (Table 1), indicates that the peptide may counteract the anti-NMDA effects of some endogenous histogranin.

Very few drugs are known to antagonize or compensate for the behavioural deficits induced by blockade or substimulation of the NMDA receptor. Such drugs include agonists of the glycine site on the NMDA receptor complex, the atypical antipsychotic, clozapine (Haber, 1993), and selective sigma receptor ligands (Maurice et al., 1994a). The fact that the physiopathology of schizophrenia reportedly involves a decrease in glutamate transmission (Kim et al., 1980) indicates that the design of compounds based on the structures of histogranin and its antagonist histogranin-(1–10), which interact non-competitively with the NMDA receptor, may find an application in the management of some cognitive disorders.

## Acknowledgements

Thanks are due to Jean Bayle for building the apparatus used for passive avoidance testing. This research was supported by the M.R.C. (Canada), and by I.R.M.E. and A.F.M. (France).

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