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NMDA-Induced Spinal Hypersensitivity Is Reduced by Naturally Derived Peptide Analog [Ser^1]Histogranin

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HAMA, A. T., J. B. SIEGAN, U. HERZBERG AND J. SAGEN. *NMDA-induced spinal hypersensitivity is reduced by natural peptide analog [Ser^1]histogranin*. PHARMACOL BIOCHEM BEHAV **62**(1) 67–74, 1999.—*N*-methyl-D-aspartate (NMDA) receptor activation is thought to initiate a cellular cascade of events in the spinal cord that leads to neuronal hyperactivation and exaggerated persistent pain behaviors. Previous studies have demonstrated that implantation of adrenal medullary tissue into the spinal subarachnoid space reduces abnormal pain behaviors such as hyperalgesia and allodynia, possibly by intervening in the NMDA hyperexcitability cascade. Histogranin is a 15-amino acid peptide possessing NMDA receptor antagonist activity that has been isolated from adrenal medullary tissue. The present study examined the ability of stable analog [Ser^1]histogranin to reduce abnormal pain-related behaviors induced in rats by direct activation of spinal NMDA receptors. The intrathecal injection of NMDA (5.0, 10.0, 20.0 nmol) produced significant thermal and mechanical hyperalgesia and tactile allodynia in a dose-related fashion. [Ser^1]histogranin injected intrathecally prior to NMDA injections dose dependently attenuated or completely blocked hyperalgesia and allodynia. In addition, [Ser^1]histogranin administration following NMDA-induction of abnormal pain behaviors reversed these effects. These results demonstrate that a naturally derived adrenal medullary neuropeptide can prevent and reverse NMDA-mediated spinal hyperexcitability. The distinct profile and robust activity of [Ser^1]histogranin suggest novel alternative approaches in the management of pain and other CNS disorders involving abnormal excitatory neurotransmission. © 1998 Elsevier Science Inc.

Adrenal medulla Allodynia Analgesia Hyperalgesia *N*-methyl-D-aspartate receptor Neuropathic pain Spinal cord Chromaffin cell

EXPERIMENTAL investigations into the mechanisms of intractable and abnormal pain syndromes suggest that dysfunctional or hyperexcitable central processing is in part responsible for exaggerated responses to peripheral stimuli and its persistent nature (5,11). It has been hypothesized that subsequent to peripheral injury, a barrage of excitatory neurotransmission hyperexcites postsynaptic neurons in the CNS, leading to sensitization and exaggerated responses to subsequent stimulation. Elements that are thought to be important to the initiation of this process of central sensitization are opening of *N*-methyl-D-aspartate (NMDA) receptor calcium ion channels, nitric oxide synthase activation, and the production of nitric oxide. Cellular events that occur further along the cascade include enhanced metabolic and genomic activity, and possible cell death if neuronal activation persists (5,27,29,43). Several laboratories have investigated NMDA receptor blockade as a means of halting central sensitization and the subsequent development of abnormal pain behaviors. NMDA receptor antagonists have been shown to block the development of hyperalgesia when administered prior to nerve or tissue injury and to reduce the severity of hyperalgesia when administered following injury (10,23,33,44,49,50). However, motor weakness and dysfunction is often encountered in behavioral studies (4,8,50), and significant psychomimetic and motor side ef-

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fects have been reported to occur clinically at doses of NMDA antagonists necessary to reach therapeutic levels (2,3, 13,24,42).

Adrenal medullary chromaffin cells implanted in the subarachnoid space of the lumbar spinal cord have been shown to reduce pain behaviors resulting from peripheral nerve injury, peripheral inflammation, or direct intrathecal injection of NMDA (15,36,37,39,48). Chromaffin cells of the adrenal medulla secrete numerous substances, including catecholamines and opioid peptides, which may in part be responsible for the analgesic actions of the transplants. However, neither opioid nor α-adrenergic antagonists block suppression of abnormal pain behaviors by adrenal medullary transplants in tonic or persistent pain models, suggesting that other mechanisms are responsible for chronic pain reduction by the implanted cells (36,37,39). Histogranin is a 15-amino acid peptide that has been isolated from adrenal medullary tissue and appears to possess NMDA receptor antagonist activity (20,30,35). This peptide, and a stable analog, [Ser^1]histogranin (SHG), have been shown to displace NMDA receptor ligand binding in rat brain homogenates and block NMDA-induced convulsions in mice (20,35). Previous findings in our laboratory have demonstrated that intrathecally injected SHG can attenuate abnormal pain behaviors following peripheral nerve injury and the tonic phase of the formalin pain response at doses with no apparent motor side effects (38,41). In addition, recent findings have demonstrated nonopioid antinociceptive activity of systemic and ICV histogranin and related peptides in the mouse writhing assay (19). The goal of the present study was to determine whether this naturally derived adrenal medullary peptide reduces spinal hypersensitivity induced by direct activation of spinal NMDA receptors. A preliminary report of these findings has been presented previously (40).

METHOD

Animals

All procedures were approved by the Animal Care Committee at the University of Illinois at Chicago, and followed the guidelines in the NIH Guide for the Care and Use of Laboratory Animals. Male Sprague–Dawley rats (325–375 g; Sasco, WI) were used.

Surgical Procedures

Rats were implanted with indwelling intrathecal catheters as described in previous studies (36). Animals were anesthetized (Nembutal, 40 mg/kg, IP, supplemented as necessary). Using aseptic surgery, the atlanto-occipital membrane was exposed, a slit in the membrane was made, and a PE-10 catheter was threaded down the subarachnoid space, the tip terminating at the upper level of the lumbar enlargement The catheter was secured to the occipital bone by securing a knot covered with dental acrylic with cyanoacrylate. The incision was closed with wound clips and animals returned to their cages for observation. Animals were housed individually and allowed food and water ad lib. Rats were allowed 1 week to recover before testing. Any animals displaying neurological impairment during this recovery period were euthanized.

Drugs

[Ser^1]-histogranin (SHG) was custom synthesized by Research & Diagnostic Antibodies (Berkeley, CA). Peptide purity was assessed using HPLC and determined to be >95%. *N*-methyl-D-aspartate (NMDA) was obtained from Sigma Chemical Co. (St. Louis, MO). Both SHG and NMDA were dissolved in normal saline prior to use.

Behavioral Testing

Behavioral responsiveness to noxious and nonnoxious stimuli were assessed as described in previous studies in our laboratory (15,36). Behavioral observations were not routinely done blind; thus, some experimenter bias cannot be eliminated. To measure responses to a noxious heat stimulus, rats were placed on an elevated glass surface and a radiant heat stimulus was aimed at the plantar surface of the hindpaw (16). The time between the onset of the stimulus and the rat's hindpaw withdrawal from the stimulus was recorded as the withdrawal latency, measured in seconds. Three trials per hindpaw were averaged for statistical analysis. To measure responses to a noxious mechanical stimulus, a paw pressure apparatus (Ugo-Basile) was used. Increasing pressure (rate of 64 g/s) was applied to the ventral surface of the animals hindpaws. The test terminated when the rat withdrew its hindpaw and the scale value was recorded as the withdrawal threshold. The withdrawal thresholds of left and right hindpaws were averaged. To measure sensitivity to innocuous touch, a series of calibrated von Frey filaments (range 3.6–76 g) were used . Animals were placed on an elevated wire mesh surface and filaments were applied to the plantar surface of the hindpaw. Enough pressure was used to bow the filaments that were applied five times at a rate of about 2/s. The withdrawal threshold (g) was reached when a filament evoked a brisk withdrawal reflex. Testing was alternated between both hindpaws and thresholds from both were averaged.

Experimental Protocol

After baseline assessment, SHG (1.0 or 4.0 μg) or saline vehicle was intrathecally injected in a volume of 15 μl followed by a flush with 10 μl of saline. This dose range has previously been shown to attenuate neuropathic pain behaviors following peripheral nerve injury producing abnormal motor behaviors or impairment (38,41). Fifteen minutes following SHG or saline injection, NMDA (5.0, 10.0, or 20.0 nmol in 15 μl) was intrathecally injected. Responses to noxious and innocuous stimuli were again assessed at 5, 15, 30, 45, and 60 min after NMDA injections. Intrathecal injection of NMDA has been shown in our laboratory and others to produce a transient robust hyperalgesia, presumably by direct activation of spinal NMDA receptors (1,21,26,36). Each animal was utilized for three drug/dose combinations (n = 12 animals/group), with a minimum interval of 72 h between intrathecal injections. To assess the effects of SHG alone on these behavioral tests, an additional small group of animals (n = 6/group) received intrathecal injections of SHG alone (1.0 or 4.0 μg) or saline vehicle, and tested at 15, 30, and 60 min following injection.

To assess the effectiveness of [Ser^1]histogranin in reducing ongoing hyperexcitation, a separate group of animals received intrathecal SHG (1.0 μg) or saline after intrathecal NMDA (10.0 nmol) injections (n = 10 animals/group). Animals were tested at 5 and 15 min following NMDA to establish thermal and mechanical hyperalgesia and tactile allodynia. Immediately following the 15-min assessment, animals received SHG or saline and were again assessed at 30, 45, and 60 min.

Statistical comparisons between treatment groups were done using ANOVA for repeated measures and the New-

man–Keuls test for multiple post hoc comparisons (SigmaStat, Jandel Scientific). When possible, estimations of relative NMDA potency ratios were calculated using statistical software and computer-generated regression lines (46). To do this, log dose–response curves at 30 min (time of peak effect) following the intrathecal injection of the different doses of NMDA were generated following pretreatment with saline or SHG (1.0 and 4.0 μg). If possible, parallel lines were constructed by the program, and the potency of NMDA to produce hyperalgesia following SHG was calculated relative to the saline vehicle.

RESULTS

Effects of [Ser^1]Histogranin on Pain Behaviors

To assess whether SHG alone produced any effects on thermal and mechanical responses, a small group of animals were tested for baseline responses and then received intrathecal injections of either [Ser^1]histogranin (1.0 or 4.0 μg) or saline. Results are shown in Fig. 1. Neither SHG dose produced significant alterations in responses to noxious or innocuous stimuli compared to saline [overall $F(2, 6) = 2.1, 0.48, 0.49$, respectively for thermal hyperalgesia, mechanical hyperalgesia, and tactile allodynia, respectively; $p > 0.05$]. SHG did appear to produce slight thermal hyperalgesia, particularly at the 1.0 μg dose at 30 min postinjection (Fig. 1A; $p < 0.05$ compared to baseline), but this was not statistically significant compared to saline vehicle.

Thermal Hyperalgesia

Figure 2 shows alterations in withdrawal latencies to a noxious thermal stimulus in animals receiving intrathecal injections of [Ser^1]histogranin (1.0 or 4.0 μg) or saline 15 min prior to NMDA (5.0 nmol, Fig. 2A; 10.0 nmol, Fig. 2B; 20.0 nmol, Fig. 2C). At all doses of NMDA, withdrawal latencies were significantly decreased in saline-treated animals ($p < 0.05$ compared with baseline). In these saline-pretreated animals, maximal hyperalgesia was observed 30 min after IT NMDA and gradually diminished towards pre-NMDA baselines over 60 min. The time course and magnitude of thermal hyperalgesia following intrathecal NMDA injections was similar to that reported previously in our laboratory (36).

Pretreatment with intrathecal injections of [Ser^1]histogranin significantly attenuated NMDA-induced hyperalgesia at all three NMDA doses [overall $F(2, 10) = 38.3, 35.6, 61.6$; $p < 0.001$, for 5.0, 10.0, and 20.0 nmol, respectively]. SHG attenuated the thermal hyperalgesia in a dose-related fashion. The lower dose of SHG (1.0 μg) completely eliminated thermal hyperalgesia by 5.0 nmol NMDA (Fig. 2A; $p > 0.05$ compared to baseline), while partially attenuating the thermal hyperalgesia resulting from higher doses of NMDA (Fig. 2B and C; $p < 0.05$ compared to both baseline and saline pretreated animals at the 30-min peak following NMDA injection). In contrast, the higher dose of SHG (4.0 μg) completely blocked NMDA hyperalgesia, even following 20 nmol intrathecal NMDA (Fig. 2C; $p > 0.05$ compared to baseline). The higher dose of SHG produced significantly greater suppression of NMDA-induced thermal hyperalgesia at all three doses of NMDA ($p < 0.05$ compared to the lower dose of SHG). Interestingly, at 5 nmol NMDA, 4.0 μg SHG produced a transient but statistically significant thermal hypoalgesia (Fig. 2A; $p < 0.05$ compared to baseline). Estimated potency ratios for NMDA were 4.6 and 14.9 for saline-pretreated compared to 1.0 and 4.0 μg SHG-pretreated animals, respectively.

Mechanical Hyperalgesia

Decreased withdrawal thresholds to noxious mechanical pressure was observed within 5 min after NMDA injection in saline pretreated animals (Fig. 3; $p < 0.05$ compared to baseline for all three doses), and followed a time course similar to that obtained for thermal hyperalgesia, with peak mechanical hyperalgesia occurring 30 min following intrathecal NMDA, and recovering towards baseline by 60 min.

In contrast, animals pretreated with either 1.0 or 4.0 μg SHG displayed attenuated hyperalgesia to noxious pressure [overall $F(2, 10) = 37.18, 31.1, 27.1$; $p < 0.001$ for 5.0, 10.0, and

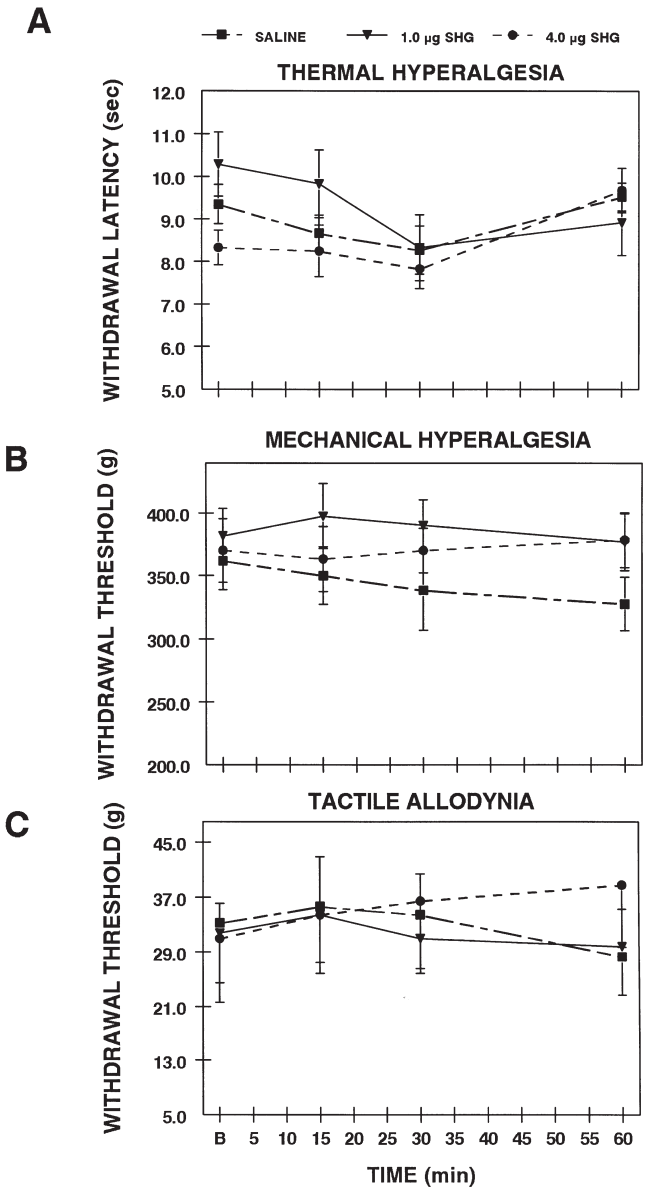

FIG. 1. Time course of responses to noxious thermal (A), noxious mechanical (B), and innocuous tactile (C) stimuli following intrathecal injections of [Ser^1]histogranin (SHG) at 1.0 μg (triangles) or 4.0 μg (circles) doses or saline vehicle (squares). Data are presented as the mean ± SEM. *Indicates $p < 0.05$ compared to saline. $n = 6$ animals/group.

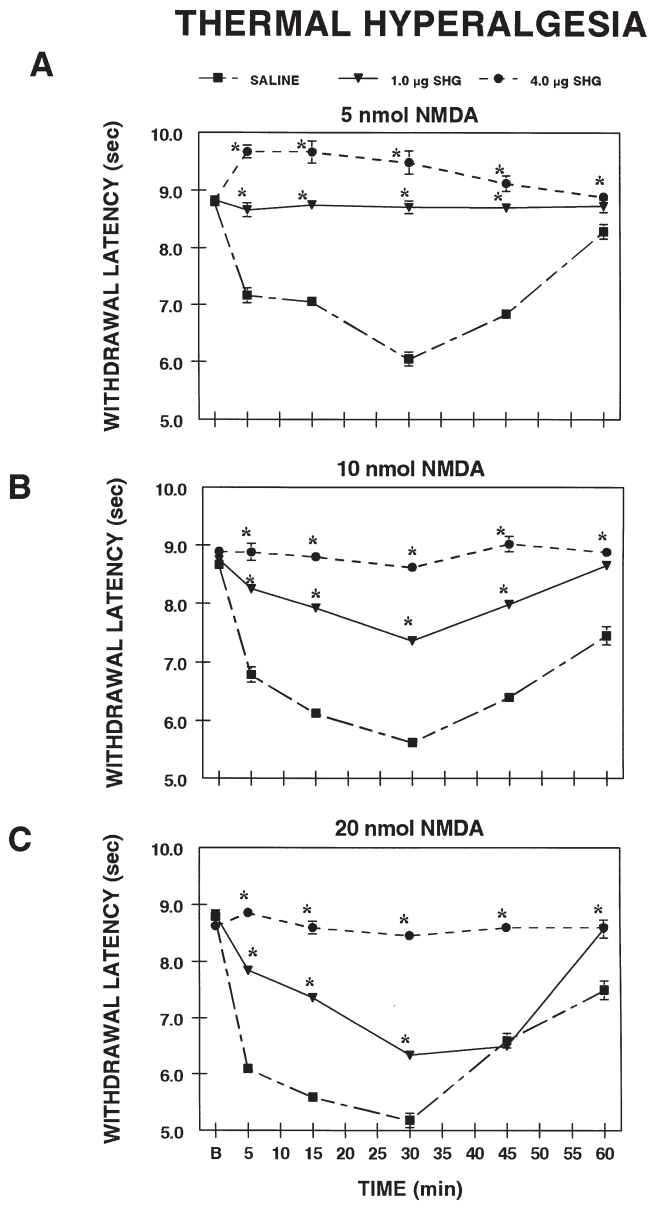

FIG. 2. Time course of response to noxious heat following intrathecal injection of 5.0 (A), 10.0 (B), and 20.0 (C) nmol NMDA. Baseline (B) response latencies were measured prior to intrathecal injection of [Ser[1]]histogranin (SHG) or saline. NMDA was intrathecally injected 15 min after intrathecal SHG (1.0 μg, triangles; 4.0 μg, circles) or saline (squares). The ordinate is paw withdrawal latency (seconds) to noxious heat. The abscissa is time (minutes) after intrathecal NMDA injection. In this and subsequent figures, data are presented as the mean ± SEM and standard error bars are included on all graphs. Occasionally, standard error bars are smaller than the symbol. *Indicates $p < 0.05$ compared to saline. $n = 12$ animals/group.

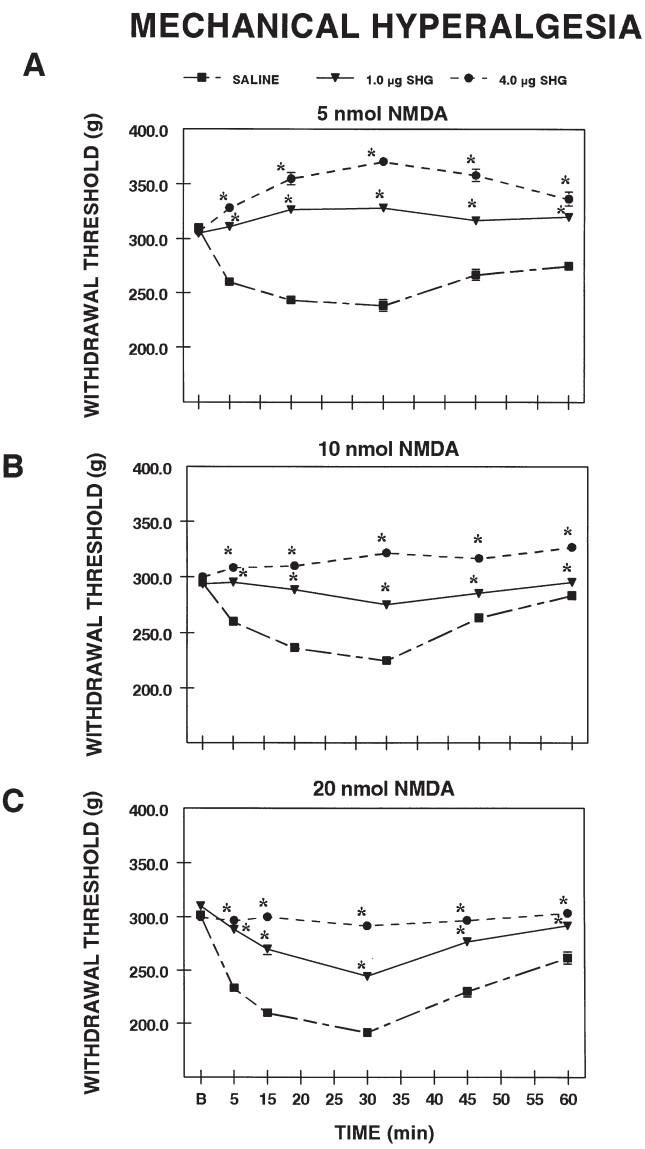

FIG. 3. Time course of response to noxious pressure following intrathecal injection of 5.0 (A), 10.0 (B), and 20.0 (C) nmol NMDA. Baseline (B) response thresholds were measured prior to intrathecal injection of [Ser[1]]histogranin (SHG) or saline. NMDA was intrathecally injected 15 min after intrathecal SHG (1.0 μg, triangles; 4.0 μg, circles) or saline (squares). The ordinate is paw withdrawal threshold (g) to increasing pressure. The abscissa is time (minutes) after intrathecal NMDA injection. Data are presented as the mean ± SEM. *Indicates $p < 0.05$ compared to saline. $n = 12$ animals/group.

20.0 nmol NMDA, respectively]. Following 5.0 nmol NMDA, responses to noxious pressure were similar to baseline in animals that received 1.0 μg SHG (Fig. 3A; $p > 0.05$ compared to baseline). As observed with noxious thermal stimulation, increased withdrawal thresholds to noxious mechanical stimulation suggestive of hypoalgesia were observed in animals receiving 4.0 μg SHG ($p < 0.05$ compared to baseline). At 10 nmol NMDA, animals that received 1.0 μg SHG displayed normal withdrawal responses to noxious pressure, except at the 30 min. NMDA peak, when a modest hyperalgesia was observed (Fig. 3B). This modest hyperalgesia was significantly attenuated compared to that observed in saline-pretreated animals ($p < 0.05$). A more rapid and robust mechanical hyperalgesia was observed following 20 nmol NMDA in animals receiving 1.0 μg SHG (Fig. 3C; $p < 0.05$ compared to baseline), although this response was significantly reduced compared to

saline-pretreated animals ($p < 0.05$). In animals pretreated with 4.0 μg SHG, no hyperalgesia was observed at any dose of NMDA at any time ($p > 0.05$, compared to baseline). Similar to responses to noxious thermal stimulation, attenuation of mechanical hyperalgesia appeared to be dose related, with significantly greater effects observed with the higher dose of SHG ($p < 0.05$ compared to 1.0 μg SHG at all three NMDA doses). Estimated potency ratios for NMDA-induced mechanical hyperalgesia were 3.5 and 8.5 for saline-pretreated compared with SHG-pretreated (1.0 and 4.0 μg, respectively)-pretreated animals.

Tactile Allodynia

Figure 4 shows responses to innocuous tactile stimuli following intrathecal injections of NMDA. In saline-pretreated animals, tactile allodynia was observed following all three doses of NMDA within 5 min following intrathecal injection ($p < 0.05$ compared to baseline). The decrease in withdrawal thresholds persisted throughout the observation period, although some recovery towards baseline was observed at 60 min. post-NMDA.

NMDA-induced tactile allodynia was significantly attenuated by pretreatment with SHG (overall $F(2, 10) = 17.2, 23.0$, and 39.8 for 5.0, 10.0, and 20.0 nmol NMDA, respectively). Tactile allodynia following 5.0 nmol NMDA was completely blocked in animals receiving either 1.0 or 4.0 μg of SHG (Fig. 4A; $p > 0.05$ compared to baseline). At higher doses of NMDA, tactile allodynia was observed in animals pretreated with 1.0 μg SHG-pretreated animals, the effect being maximal at 30 min post-NMDA ($p < 0.05$ compared to baseline). However, both the magnitude and time course of tactile allodynia were reduced in these animals compared to saline pretreated animals (Fig. 4B and C). Tactile allodynia was significantly attenuated compared to saline-pretreated animals at all time points except 30 min following the highest NMDA dose. When the higher dose of SHG was used (4.0 μg), tactile allodynia was completely eliminated at all doses of NMDA ($p > 0.05$, compared to baseline).

Effect of [Ser^1]Histogranin After Induction of Hyperexcitation

To assess the ability of SHG to reverse hyperexcitation once initiated by intrathecal NMDA, a separate group of animals received SHG injections 15 min following NMDA. Results are shown in Fig. 5. As in saline-pretreated animals described above, intrathecal injections of 10 nmol NMDA produced significant thermal and mechanical hyperalgesia (Fig. 5 A and B) and tactile allodynia (Fig. 5C) within 5 min following injection ($p < 0.05$ compared to baseline for all three tests). Following determination of hyperalgesia and allodynia 15 min post-NMDA, animals received 1.0 μg SHG or saline intrathecally (at arrows). In saline-injected animals, hyperalgesia and allodynia remained apparent at 30–60 min following NMDA injection, tending towards baseline following the 30-min peak NMDA effects. In contrast, SHG reversed the NMDA-induced hyperalgesia and allodynia [overall $F(1, 10) = 52.5$, 31.8, and 42.1 for responses to noxious thermal, noxious mechanical, and innocuous tactile stimuli, respectively). The [Ser^1]histogranin completely reversed the hyperalgesia and allodynia to pre-NMDA baseline responses within 15 min following injection ($p > 0.05$ compared to baseline for all three tests). There was no reoccurrence of hyperalgesia or allodynia throughout the remainder of the observation period.

DISCUSSION

Results of this study demonstrate that intrathecal application of a naturally derived peptide with NMDA antagonist properties can attenuate spinal hyperexcitability induced by direct intrathecal injection of NMDA. The stable analog of histogranin, [Ser^1]histogranin, exhibited both preemptive as

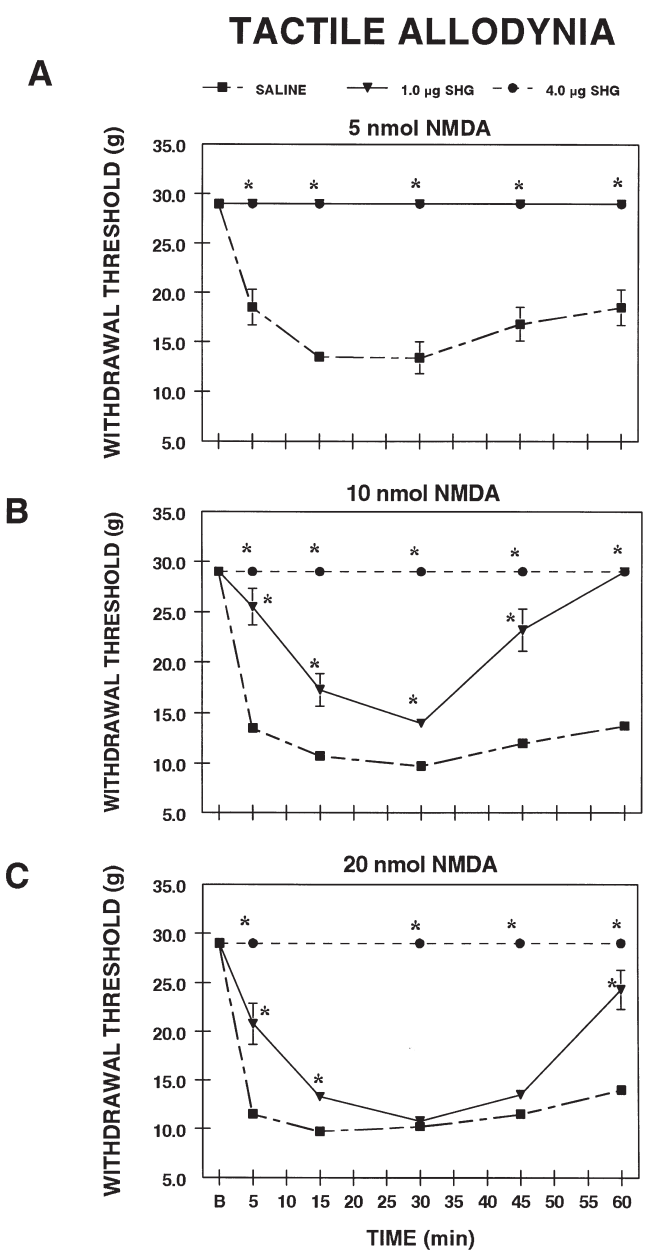

FIG. 4. Time course of response to innocuous touch following intrathecal injection of 5.0 (A), 10.0 (B), and 20.0 (C) nmol NMDA. Baseline (B) response thresholds were measured prior to intrathecal injection of [Ser^1]histogranin (SHG) or saline. NMDA was intrathecally injected 15 min after intrathecal SHG (1.0 μg, triangles; 4.0 μg, circles) or saline (squares). The ordinate is paw withdrawal threshold (g) to calibrated von Frey filaments. The abscissa is time (minutes) after intrathecal NMDA injection. *Indicates $p < 0.05$ compared to saline. Data are presented as the mean ± SEM. $n = 12$ animals/group.

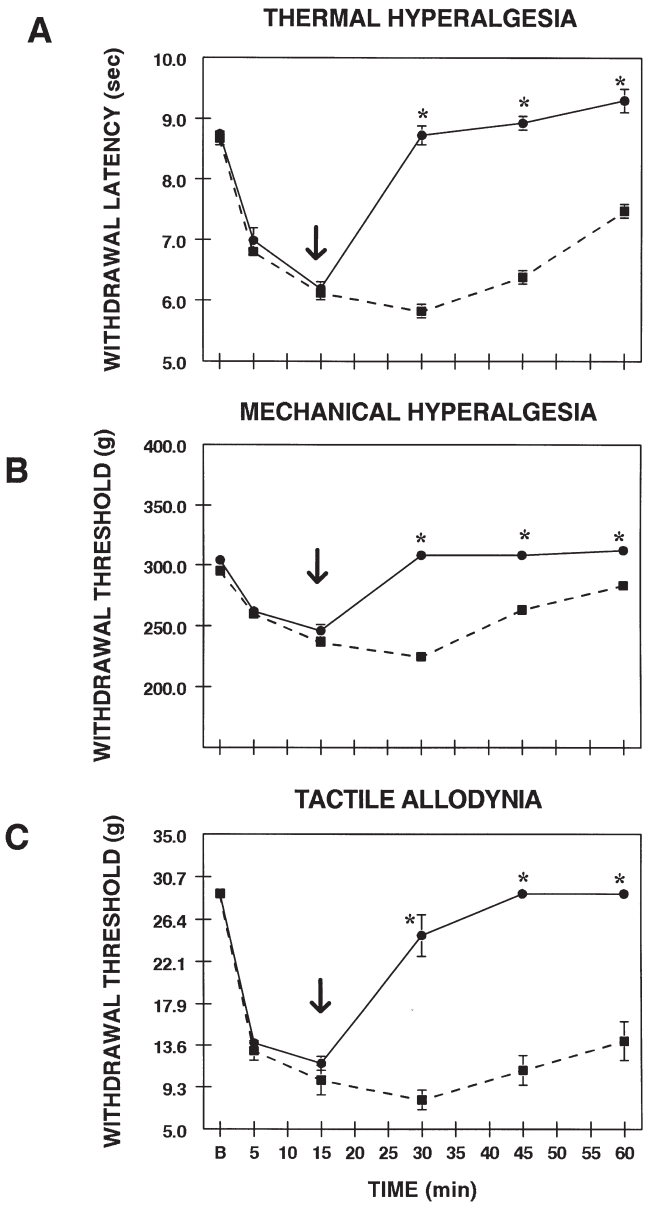

FIG. 5. Time course of effects of intrathecal injection of [Ser[1]]histogranin after intrathecal injection of 10.0 nmol NMDA. Baseline (B) response thresholds to noxious heat (A), noxious pressure (B), and innocuous touch (C) were measured prior to intrathecal injection of NMDA. [Ser[1]]histogranin (1.0 μg, circles) or saline (squares) was intrathecally injected 15 min after intrathecal NMDA (at arrow). The ordinate is latency or threshold response to a stimulus. The abscissa is time (minutes) after intrathecal NMDA injection. *Indicates $p < 0.05$ compared to saline. Data are presented as the mean ± SEM. $n = 10$ animals/ group.

well as therapeutic properties, as sufficient doses could both block and reverse exaggerated responses to noxious and innocuous stimuli.

Histogranin, a 15-amino acid peptide, was originally isolated from the bovine adrenal medulla and localized to the secretory granule fraction (20). Immunoassays also revealed a high concentration of this peptide in the pituitary gland, with low but significant levels in the brain and blood plasma. This natural peptide and its stable synthetic analog SHG produce a dose-dependent protection against ICV NMDA-induced convulsions in mice, but not against convulsions induce by other excitatory amino acid agonists (20,35). Excitatory amino acid receptor stimulation has also been reported to enhance nociceptive responsiveness (1,18,22,36), and activation of the NMDA receptor appears to be a key event in the initiation of spinal neuron hyperexcitability and subsequent abnormal pain responses (5,11). This initiation of abnormal chronic pain processes in the spinal cord may be mimicked by the direct intrathecal injection of NMDA. Intrathecally administered NMDA produces behavioral evidence of transiently increased pain sensitivity including thermal hyperalgesia and exaggerated responsiveness to light touch (1,6,18,21,31,36).

The present findings demonstrate that putative NMDA antagonist peptide SHG can block the initiation of NMDA-evoked pain responses in a dose-related fashion. Similarly, pretreatment with both competitive (D-2-amino-5-phosphonovalerate; APV) and noncompetitive (MK-801) NMDA antagonists have been shown to block the hyperalgesic effects of intrathecal NMDA (1,21,25,31). Previous findings in our laboratory have also indicated that intrathecal SHG pretreatment attenuates the second phase pain behaviors of the formalin response (38), which is thought to be initiated by activation of NMDA receptors, and is also suppressed by other NMDA antagonists (4,7,9,14,17,28,47,51). Together, these findings suggest that the peptide SHG provides NMDA antagonist-like activity in preventing the initiation of exaggerated pain behaviors.

The present study also demonstrated that SHG can reverse hyperalgesia and allodynia after induction by intrathecal NMDA. This contrasts with effects of intrathecally administered MK-801, which fails to reverse thermal hyperalgesia once it is initiated by NMDA (21). In addition, while prophylactic administration of both competitive (e.g., APV and CPP) and noncompetitive (e.g., MK-801) NMDA receptor antagonists greatly suppress or delay the onset of hyperalgesia following peripheral nerve injury or inflammation (10,12), the effects of these agents given after injury are contradictory, with some laboratories reporting decreased hyperalgesia and others reporting no effect (7,12,23,32,33,44,50). In support for the present findings demonstrating reversal of NMDA-induced pain behaviors, intrathecal SHG has also been shown to transiently reduce established hyperalgesia and allodynia in animals with peripheral nerve injury (41). Together, these results suggest that SHG can reverse, as well as prevent, NMDA-mediated spinal hyperexcitability.

A unique site of action may account for the unique profile and robust activity of SHG in blocking and reversing NMDA receptor activation. That SHG has an activity profile distinct from other NMDA antagonists is also suggested by its ability to reverse allodynia, in contrast to other NMDA antagonists (45,49). The precise pharmacologic mechanisms of SHG are unclear, and the data thus far do not completely support activity at the classical NMDA receptor sites (34). Histogranin and SHG noncompetitively inhibit binding of [^{3}H]CGP 39653, a ligand for the glutamate site on the NMDA receptor, in a dose-dependent manner in rat brain membranes (20,35). However, high affinity saturable binding sites of SHG in rat brain membranes is not altered by specific ligands of the various binding domains on the NMDA receptor complex, including NMDA, phencyclidine, and glycine (34). Polyamine site-specific agonists and antagonists displace SHG binding, but its noncompetitive nature and the failure of SHG to alter

polyamine binding indicate that the histogranin receptor is also distinct from the polyamine site (34). The authors suggest that histogranin acts as an endogenous NMDA antagonist via binding to a unique domain located on or near the NMDA receptor complex.

In addition, it is not clear whether histogranin acts as a pure NMDA antagonist. Interestingly, the combination of SHG with low doses of NMDA produced hypoalgesia, particularly to noxious thermal stimuli compared to preinjection baselines. This cannot be explained by intrinsic antinociceptive effects of SHG, because intrathecally injected SHG alone did not alter responses to mechanical pressure or light touch and produced a slight hyperalgesia to noxious heat. However, in chronic pain states such as that following peripheral nerve injury in the CCI model, the antihyperalgesic effects of SHG predominate (41). Thus, histogranin may possess both antagonist and agonist properties in its interaction with the NMDA receptor complex.

The effects of adrenal medullary implants in the spinal subarachnoid space on NMDA-induced spinal hypersensitivity are similar to that observed with intrathecal SHG in the present study (36). The adrenal medulla manufactures and secretes numerous substances, including opioid peptides and catecholamines, which may play a role in reducing pain via interaction with spinal opiate and α-adrenergic receptors. However, attenuation of NMDA-induced hyperalgesia and allodynia in adrenal medullary implanted animals is not reversed by opioid or α-adrenergic antagonists. Adrenal medullary implants also suppress the second phase of the formalin response and neuropathic pain behaviors following peripheral nerve injury, effects that are not completely blocked by opioid or α-adrenergic antagonists (37,39). These findings suggest the possibility that other agents produced by the implanted cells, such as histogranin, may be involved in pain alleviation.

In summary, the results of this study demonstrate that [Ser^1]histogranin, an analog of a natural peptide produced by the adrenal medulla, can reduce or eliminate abnormal pain-related behaviors brought about by NMDA-mediated spinal activation. The distinct profile and robust activity of SHG in blocking and reversing NMDA activation, together with findings from binding studies, suggest a unique pharmacologic site of action. These naturally derived peptides may lead to novel alternative approaches in the management of pain and other CNS disorders involving abnormal excitatory neurotransmission.

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