Inhibition by the chromaffin cell-derived peptide serine-histogranin in the rat's dorsal horn

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Abstract

The heptadecapeptide histogranin, synthesized by adrenal chromaffin cells, is implicated in the analgesia produced by transplanting chromaffin cells into the spinal cord, including block of hyperalgesia mediated by NMDA-subtype glutamate receptors. To examine the neurophysiological basis for this analgesia, we applied the stable analog [Ser^1]-histogranin (SHG) by iontophoresis near extracellularly recorded wide-dynamic range (WDR) neurons in anesthetized rats. When SHG was applied during peripheral electrical stimulation of A and C fibers at 0.1 Hz, the C-fiber response was significantly inhibited but the A-fiber response was unaffected. SHG also opposed the NMDA-receptor-dependent post-tetanic facilitation (wind-up) of C-fiber responses produced by increasing the rate of peripheral afferent stimulation to 1 Hz for 20 s. To test whether block of NMDA-subtype receptors could be wholly or partially responsible for this suppression, SHG was applied during sequential pulsed iontophoresis of three agonists targeting distinct excitatory synaptic receptors: NMDA, kainate and substance P. All three excitatory effects were reversed by SHG; this reversal outlasted the 10–30 min observation period when higher SHG doses were applied (>60 nA). Histogranin therefore probably produces prolonged spinal analgesia by opposing the basal and potentiating synaptic effects of C-fibers on dorsal horn neurons. Actions besides or in addition to NMDA-receptor antagonism (e.g., agonism at inhibitory postsynaptic receptors or block of voltage-gated cation channels on C-fibers) are implied by the diversity of excitatory transmitters opposed by SHG.
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Adrenal chromaffin cells synthesize a large number of releasable neuroactive substances, including several with known analgesic actions [22], and their transplantation into the rat's spinal subarachnoid space leads to long-term analgesia [13]. Both catecholamines and met-enkephalin are released into the subarachnoid space from chromaffin cell transplants and appear to play a role in the consequent analgesia [14–16,21]. However, components of an NMDA-mediated cascade that initiates central pain sensitization are also suppressed by adrenal medullary transplants, including nitric oxide synthase [6], cyclic guanosine monophosphate [19] and c-fos protein [17,20]. One substance released from chromaffin cells is known to have a fairly selective affinity for NMDA subtype receptors, namely the heptadecapeptide histogranin [9,11]. Its stable analog, [Ser^1]-histogranin (SHG), if administered intrathecally, blocks the hyperalgesia

and allodynia produced in rats by intrathecal NMDA [7]. SHG can non-competitively inhibit the binding of NMDA receptor antagonists in rat membrane preparations, possibly by acting at the regulatory polyamine on the receptor [11,18].

The mechanism of histogranin's spinal analgesic action has not previously been analyzed by electrophysiological recording. In the present experiments, we examined wide-dynamic range (WDR) mechanoreceptive neurons in the dorsal horn of halothane-anesthetized rats, which convey ascending signals that are important in the perception of pain [1]. Rapid repetitive stimulation of the C-fiber input to WDR cells produces a homosynaptic post-tetanic potentiation (wind-up) that requires activation of NMDA-subtype glutamate receptors [5,23]. We had previously shown that wind-up is suppressed by adrenal medullary transplants [8]. Here we explore how iontophoresis of SHG near WDR neurons affects basal and wind-up responses to peripheral electrical stimulation, and whether this substance's actions can be entirely accounted for by antagonism at NMDA receptors.

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Experimental procedures were carried out on female, Sprague-Dawley rats weighing 250–350 g ($n = 14$). All research on animals was approved by a local Institutional Animal Care and Use Committee (University of Illinois College of Medicine at Rockford, #375-02). In 11 rats, anesthesia was induced with 3% halothane in oxygen inside a closed Plexiglass chamber. A rapid ventral neck incision allowed insertion of a tracheal tube, and halothane was then applied through this tube at a rate of 0.2–0.5 l/min and a concentration of 1.1–1.3%. Sodium pentobarbital was employed as the anesthetic in 3 animals to verify that certain responses were not due to halothane; induction by 50 mg/kg (i.p.) pentobarbital was followed by continuous intravenous infusion of 10 mg/kg/h. In all animals, the external jugular was cannulated, and a syringe pump delivered Ringers solution intravenously at a rate of 1–2 ml/h. A pulse oximetry probe was used to monitor blood oxygen saturation. A direct-current electric heating blanket, controlled by the feedback signal from a rectal thermistor kept the body temperature at 37 °C. A laminectomy was performed over the T11 to L2 vertebrae, corresponding to T13 to S1 spinal segments. Spines rostral and caudally to the laminectomy were held by clamps to prevent motion. The dura was cut over the target spinal segments and the area covered by solidified agar (5%, dissolved in 0.9% saline) to further minimize tissue movement.

Recordings were made with multi-barrel micropipettes (5 or 7 barrels). These were fabricated from glass tubing with glass fibers inside (obtained from World Precision Instruments). The central tube was used for recording action potentials, and was filled with 3 M NaCl. Another barrel contained 2 M NaCl in which 2.5% fast-green dye had been dissolved, to act as a return current path for marking locations histologically. [Ser[1]]-histogranin (SHG) was obtained as its trifluoroacetate salt from Bachem (Torrance, CA) at >94% purity, and all other substances were obtained from Sigma/RBI (Natick, MA). SHG was dissolved at a concentration of 1.25 mM in 165 mM NaCl and its pH was adjusted to 5.5 with NaOH. A vehicle control was prepared by starting with 165 mM NaCl and adjusting its pH with NaOH to 5.5. NMDA, kainate and glycine were separately dissolved at 50 mM in Tris buffer at pH 8 and applied by iontophoresis as anions. Spermidine (*N*-[3-aminopropyl]1,4-butanediamine) and substance P were dissolved in Tris buffer at pH 4.5, 10 mM, and applied as cations. Iontophoresis employed isolated constant-current sources (±100 V compliance, World Precision Instruments model 260). Since only 2 cells were studied with glycine, its effects are not included below.

The L4 dorsal horn was sampled from 0.2 to 0.8 mm lateral to the midline, and 0.0 to 0.8 mm below the dorsal surface. Filtered (0.1–10 kHz) extracellular recordings, along with stimulus and iontophoresis markers, were stored on videotape (processed by a Vetter 3000A PCM adaptor). Spike times were extracted off-line by voltage–time discrimination using a dedicated time/amplitude window discriminator and event detector (Dagan WD2). Overlapped oscilloscope traces were monitored to ensure exclusion of additional units when >1 spike shape was present. Wide-dynamic-range (WDR) nociceptive neurons were distinguished from low-threshold mechanoreceptors by means of flexible von Frey probes that delivered forces which were innocuous (2 mN/mm^2) or noxious (20 mN/mm^2). The WDR neurons were defined by an increase in firing when the stimulus became noxious. In some experiments, their receptive fields were activated by subcutaneous needle electrodes, placed 1 cm apart in the receptive field, delivering 1-ms pulses at a basal rate of 0.1 Hz. The numbers of spikes was counted in time windows 0–20 ms, 40–300 ms and 300–1000 ms, respectively, representing the A-fiber, C-fiber and "post-discharge" responses [5]; the post-discharge occurred only during post-tetanic facilitation (wind-up). The stimulus amplitude (10–40 V) was adjusted to give a mean response of 3–5 action potentials in the C-fiber window. To produce wind-up, the stimulus rate was raised to 1 Hz, with at least a 2-min interval between tests. Wind-up was quantified as a fractional increase in the average response in the last five stimuli over the first five stimuli in the 20 s (1 Hz) train.

At the end of a given vertical trajectory, pressure (5 p.s.i.) was applied for 5 min to eject the fast green dye solution. Rats were sacrificed by an intravenous infusion of pentobarbital (100 mg/kg/min for 2 min), and, when breathing and all other reflexes had ceased, received intracardial perfusion of phosphate-buffered saline (pH 7.6, 4 °C) followed with 10% formaldehyde (pH 7.0). The spinal cord was removed, maintained overnight in formaldehyde, then embedded in agar and sliced in the coronal plane on a Vibratome at 50 μm thickness. Sections were stained with cresyl violet and scanned under a light microscope for dye marks. Recording positions with respect to laminar location in gray matter were verified from these marks in conjunction with micromanipulator readings.

Statistical assessment employed commercial software (SPSS 14.0). Changes in A-fiber and C-fiber responses (number of impulses) at different times relative to SHG delivery were assessed by multi-way analysis of variance (ANOVA), at a significance level of $P < 0.05$ (two-sided). Significant effects on individual neurons were found by post hoc contrasts, with Bonferroni adjustments made to significance levels. For wind-up, the change in spike numbers was analyzed similarly, with post-discharge responses also included as a dependent variable along with A-fiber and C-fiber responses. Analysis of the effect of SHG on responses to excitatory agonists was also subjected to ANOVA, taking as the dependent variable the spike count recorded during the last half of the agonist's application period plus the 5 subsequent seconds. The minimum percentage of neurons in the underlying target population responding to a particular intervention was estimated (at $P < 0.05$) by applying the equation for the binomial distribution to the proportion of sampled neurons showing significant responses (responses were always in the same direction).

All cells studied ($n = 27$) were of the WDR type and were found at depths of 418–753 μm, which was within or near lamina V according to histological analysis. The effect of SHG on responses to cutaneous electrical stimulation was studied under halothane anesthesia, by applying SHG iontophoretically for 1 min. During basal stimulation (0.1 Hz), application of SHG (60–100 nA) caused the response to C-fiber input to fall significantly ($P < 0.001$, $n = 8$ cells) as shown in Fig. 1. No significant effect was seen in the mean A-fiber response, and the iontophoresis of a vehicle control was without significant effect. Given