Spinal Strychnine Alters Response Properties of Nociceptive-Specific Neurons in Rat Medial Thalamus

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Sherman, Stephen E., Lei Luo, and Jonathan O. Dostrovsky. Spinal strychnine alters response properties of nociceptive-specific neurons in rat medial thalamus. J. Neurophysiol. 78: 628–637, 1997. Experiments in both conscious and anesthetized animals indicate that intrathecal (i.t.) strychnine (STR; glycine receptor antagonist) produces acute, reversible allodynia, as evidenced by inappropriate behavioral and autonomic responses to cutaneous tactile stimuli. Although STR is known to produce disinhibition of afferent input to the spinal cord, changes in spinal reflexes cannot fully explain the complex behaviors observed following i.t. STR. Which supraspinal sites are involved in STR-dependent allodynia and how this abnormal somatosensory message is relayed to these sites remain to be determined. The medial thalamus contains many nociceptive-specific (NS) neurons and is believed to be involved in mediating the affective-motivational aspects of pain. It is thus important to determine whether spinally administered STR elicits changes in the responses of medial thalamic NS neurons. Extracellular single-unit recordings were conducted in urethan-anesthetized rats (290–490 g). A detailed characterization of 20 thalamic NS units (1 per rat; 2 in 1 case) was conducted before and immediately after i.t. STR (40 μg). Initially, all of the units in this study were classified as NS, because they were excited by noxious pinch but not by innocuous tactile stimuli. After i.t. STR, all (formerly NS) units exhibited significant responses to innocuous tactile stimuli (brush and/or air jet) applied to lumbar or sacral dermatomes. This effect of STR on thalamic NS neurons was acute and reversible. The majority of units (11 of 20) also exhibited an increase in spontaneous firing rate. Although the complete pinch receptive field (RF) could not be determined for all units, the available data indicate that the RFSs for brush stimulation after i.t. STR were substantially different from the pre-STR pinch RFSs for all but three units. The same i.t. STR injection that caused the observed changes in medial thalamus also produced allodynia, in the form of brush-evoked cardiovascular or motor responses, in 18 of the 19 rats. The ability of NS cells in medial thalamus to respond to tactile input after i.t. STR suggests that the STR lowers the threshold of nociceptive neurons that project directly and/or indirectly to medial thalamus. These observations suggest that ascending nociceptive pathways and medial thalamic structures contribute to the expression of STR-dependent allodynia.

INTRODUCTION

Allodynia is a rare but distressing symptom of neural injury or neuropathy that is characterized by the inappropriate perception of light tactile stimuli as being painful. This clinical condition has been modeled experimentally in animals by the spinal administration of strychnine (STR), a glycine receptor antagonist. Under normal circumstances, lightly touching or stroking the hair of a conscious rat or mouse elicits no more than an orientation response. However, after intrathecal (i.t.) STR, an identical tactile stimulus evokes vigorous scratching and biting of the stimulation site, vocalization, attempts to escape, and aggressive behavior (Beyer et al. 1985, 1988; Onaka et al. 1996; Sosnowski and Yaksh 1989; Yaksh 1989). All of these behavioral responses are usually elicited only by high-intensity and potentially tissue-damaging (nociceptive) stimuli, not light touch. Thus the temporary removal of spinal glycine inhibition with i.t. STR results in a disturbance of sensation, resembling the allodynia seen in neuropathic pain patients.

In addition to the pain-related behaviors observed in conscious animals, allodynia may also be demonstrated under anesthesia. Cutaneous application of brush or air jet stimulation to STR-treated, anesthetized rats evokes an abrupt motor withdrawal response with marked tachycardia and hypertension (Sherman and Loomis 1994–1996; Yaksh 1989). These responses, suggestive of extremely noxious sensory input and elicited exclusively by nociceptive stimuli in control rats (i.e., no STR), are evoked by cutaneous stimuli (not spontaneous), occur in the absence of convulsions, and are only elicited by stimulation of discrete, segmentally localized cutaneous regions near the i.t. STR injection site (brush stimulation does not evoke motor and autonomic responses after similar doses of intravenous STR) (Sherman and Loomis 1994). In view of the allodynia observed in conscious animals, these cardiovascular and motor reflexes have been interpreted as nocifensive, and because of the innocuous nature of the stimuli, as evidence of allodynia (Sherman and Loomis 1994; Yaksh 1989). These observations suggest that low-threshold mechanoreceptive afferent inputs can potentially activate spinal nociceptive neurons to produce nociceptive responses, but that these inputs are inhibited by glycineergic modulation and are thus subliminal under normal circumstances.

One possible mechanism that could explain the effects of STR would be an enhancement of the low-threshold afferent input to spinal wide-dynamic-range (WDR) neurons, although it is also possible that STR may lead to the activation of nociceptive-specific (NS) neurons by low-threshold inputs. A number of recent studies provided evidence suggesting that indeed STR may induce these changes. For example, after iontophoresis of STR into the rat spinal dorsal horn, Wilcox et al. (1996) evoked windup of WDR and low-threshold neurons with the use of repetitive natural innocuous brush stimulation. The ability of STR to facilitate low-threshold input to spinal neurons is further supported by the observation that microdialysis of STR (1 mM) into the cat dorsal horn led to an enhancement of responses to...
hair deflection, enlargement of low-threshold receptive fields (RFs), and increased afterdischarges of low-threshold, NS, and WDR neurons (Sorkin and Puig 1996). Similar results were observed in primates, where microdialysis of STR (2 mM) resulted in a significant increase in background activity of spinal WDR and high-threshold neurons, as well as a significant enhancement of unit responses to brush stimulation (Lin et al. 1994). However, it is not clear whether the concentrations of STR used in these studies are comparable with those used in the behavioral studies, and there was no possibility of correlating the observed neural changes with simultaneous behavior or nociceptive reflexes indicative of allodynia. One of the aims of the present study was to examine the effects of i.t. STR on nociceptive neurons under the same conditions present in previous studies of STR-dependent allodynia (Sherman and Loomis 1994–1996) and also to be able to correlate the observed changes in neural responses with nociceptive cardiovascular and motor reflexes.

Because STR leads to the inappropriate interpretation of a low-threshold input as noxious and to behavioral changes that likely involve cortex and probably reflect pain, it is of interest to determine which brain sites are involved in mediating these effects and how the responses of the neurons in these regions are altered. It is generally assumed that the sensory-discriminative and affective-motivational aspects of pain are processed by different regions of thalamus and cortex. The medial thalamus contains many NS neurons and has been repeatedly implicated in mediating the affective-motivational aspects of pain. In the present study we tested the hypothesis that NS neurons in the medial thalamus would start to respond to low-threshold tactile stimulation during the period when STR-induced allodynia occurs.

The current study is the first to investigate the effects of i.t. STR on the response properties of NS neurons in rat medial thalamus and to be able to relate changes in nociceptive processing with gross nociceptive reflexes. Thus the presence of allodynia was confirmed by the ability of low-threshold stimuli to evoke cardiovascular or motor responses as previously described by Sherman and Loomis (1994). These experiments provide information about supraspinal sites that may participate in the abnormal somatosensory processing that underlies allodynia. A portion of this work has already been presented as an abstract (Sherman et al. 1996).

METHODS

Animals

Male Wistar rats (Charles River, St.-Constant, Canada) weighing 290–490 g at the time of the experiment were used for all procedures. Animals were housed in an animal care facility with a 12-h light/dark cycle (lights on at 0700 h) and free access to rat chow and tap water. All experiments were conducted in accordance with the Guidelines of the Canadian Council on Animal Care and were approved by the University of Toronto Animal Care Committee.

Implantation of i.t. catheters

Rats were fitted with i.t. catheters prepared from stretched polyethylene tubing (PE-10). General anesthesia for this procedure was provided by either halothane (1–3% in oxygen; Fluothane; Wyeth-Ayerst Canada) or methohexital sodium (Brietal; 70 mg/kg ip). As previously described (Sherman et al. 1987), the catheters were filled with sterile saline, inserted through the cisterna magna into the spinal subarachnoid space, and guided 8.5 cm caudally (L1 termination). The rostral 3–5 cm of the catheter was threaded through the skin on the back of the neck and sealed with a stainless steel plug. Animals were permitted to recover for ≥3 days after surgery, and only those without signs of neurological impairment were used in the experiments. All animals received a single injection of buprenorphine (Temgesic; 0.3 mg/kg sc) at the time of surgery. Chlorohexidine acetate cream (Hibitane; Ayerst Laboratories, Montreal, Canada; antibacterial/antifungal) was applied daily to the incision for the next 3 days.

Testing of i.t. catheters

After recovery from surgery, but ≥24 h before the acute experiment, the patency of the i.t. catheter and its approximate position in the subarachnoid space were tested with the use of an i.t. injection of lidocaine. All animals received 7 μl of preservative-free, sterile 5% lidocaine hydrochloride (Xylocaine Spinal, Astra Pharma) flushed through the i.t. catheter with 10 μl of sterile saline. Animals that failed to exhibit signs of brief, reversible, unilateral or bilateral paralysis of the hindlimbs were not used for experimentation.

Acute anesthetized animal preparation

On the day of the experiment, surgical anesthesia was induced with halothane, the left jugular vein was cannulated, and anesthesia was maintained with intravenous ethyl carbamate (urethan; 10% wt/vol in saline; Sigma Chemical, St. Louis, MO). The initial urethan dose (1.1 g/kg) was infused slowly over 5–10 min as the effect of halothane declined. Throughout the experiment, anesthesia was supplemented with intravenous urethan as required. A tracheal tube was implanted in all animals and most (14 of 19) had the left carotid artery cannulated for continuous monitoring of blood pressure. Instantaneous heart rate (HR) was derived from the blood pressure tracing off-line with the use of a computer program. Body temperature was maintained at 37°C with the use of a thermostatically regulated heating blanket and a colonic probe. Atropine (0.5–1.0 mg/kg ip; Ormond Veterinary Supply) was administered to reduce respiratory secretions. All incisions and contact points with the stereotaxic frame were infiltrated with 2% lidocaine to reduce basal sensory input.

Extracellular single-unit recordings

The animal was placed in a stereotaxic frame, a craniotomy (~3 × 4 mm) was performed over the sagittal suture in the region overlying thalamus, and the dura mater was removed from the exposed region of cortex (except directly over the midsagittal sinus). With a few exceptions (see RESULTS), thalamic recordings were conducted contralateral to the site of the i.t. catheter as determined by i.t. lidocaine injection.

Extracellular recording was performed with the use of commercially available, Parylene-coated tungsten electrodes (WB300310A and WB300308A; Micro Probe, Clarksburg, MD) plated with gold and platinum to a final impedance between 0.2 and 2.5 MΩ. Standard extracellular, single-unit recording techniques were employed. Action potentials were amplified, isolated with a dual window discriminator, and observed on an oscilloscope. Unit firing rate and blood pressure were digitized and monitored on-line with a CED 1401 data acquisition system and SPIKE-2 software (Cambridge Electronic Design). A digital recording device (VB-100-B; Instru-
tech, Great Neck, NY) was combined with a video cassette recorder for data storage.

Vertical electrode penetrations through medial thalamus were carried out with the use of an electronic microdrive (Burleigh). After entry into the thalamus (4–5 mm below the surface), the electrode was advanced in small steps (5–20 μm) and unit responses were frequently assessed by brushing, touching, tapping, joint movements, and pinching. To adequately investigate the effects of spinal STR on a medial thalamic cell, the unit needed to be clearly discriminated from other units, have a stable action potential amplitude for 1 h (no electrode drift), and have an RF that included cutaneous regions expected to be affected by i.t. STR (i.e., the hindquarters).

Once a suitable unit was found, control responses to pinch and brush were determined. The degree to which the pinch RF could be mapped varied from animal to animal. As previously reported (Dostrovsky and Guilbaud 1988, 1990), some medial thalamic neurons exhibit prolonged afterdischarges following a single pinch. When cells like these were encountered, only one or two sites were tested for responses to pinch. In most cases, a crude map of the pinch RF was determined by identifying a few sites that were responsive to pinch as well as a few that were not. After the effects of pinch were determined, brush stimuli were applied in a systematic pattern over the trunk and hindquarters (e.g., Fig. 3). Because all cells studied were NS, this stimulation confirmed the absence of any response to brush.

In a few animals, the effects of an air jet stimulus were also assessed. Air jet stimulation consisted of 20 air puffs (each 50 ms in duration) delivered through a 19-gauge needle at 2 Hz. The frequency and duration of the air puffs were controlled with a stimulator (Grass Instruments) and an electronically controlled valve. The driving pressure of 30 p.s.i. (≈207 kPa) was provided over the 20-s interval immediately preceding each cutaneous stimulus with a regulated supply of compressed air. This stimulus was applied parallel to the surface of hairy skin, and had sufficient force to deflect the pelage for 1–2 cm from the tip of the needle.

Assessment of the effects of i.t. STR

i.t. saline was not given routinely as a control for i.t. STR, because the risk of “losing” a single unit was increased by handling the short segment of i.t. catheter just behind the recording site. However, saline injections (15 μl i.t.) did not have any effect on unit responses when tested in a small group of animals and never produced any allodynia-like responses when tested in several previous studies (Sherman and Loomis 1994–1996).

After control responses were determined, each animal was given a single i.t. injection of STR. STR hemisulfate (Sigma Chemical) was dissolved in 0.9% sterile saline (Astra Pharma), injected with a hand-held Hamilton microliter syringe, and flushed through the i.t. catheter with 10 μl of saline. In all but two cases, the dose of i.t. STR was 40 μg; one animal received 50 μg and another 60 μg. The concentration of the STR solution was 10 mg/ml (near saturation) to minimize the volume of the injection and consequently reduce rostrocaudal spread in the cerebrospinal fluid.

To ensure that changes in RFs and response properties were attributable to the actions of i.t. STR and not the result of sensitization from multiple pinches, control brush stimulation was always applied to the hindquarters after responses to pinch were assessed and immediately before i.t. STR administration. After i.t. STR, pinch stimuli were not applied until after (i.t. STR-dependent) unit responses to brush could be adequately characterized.

Brush stimuli were applied frequently after i.t. STR. A normal stimulation consisted of sequentially brushing 10 different sites on the hindquarters and tail (e.g., Fig. 3) over a period of 1–2 min. Although more rostral sites were tested in a few animals, the present results are consistent with those of previous studies demonstrating that the effects of i.t. STR were segmentally localized near the site of STR delivery in the lumbar spinal cord. In some cases, the brush stimulus was reapplied at a few sites (1 at a time) to clarify the boundaries of the RF; particularly if the unit was prone to afterdischarges or if the sound of the unit on the audio monitor suggested that changes in the unit firing rate were not synchronized with the application of the stimulus. Either brush or air jet was applied at 2- to 3-min intervals for up to 30 min after i.t. STR. If STR-dependent, brush-evoked responses continued after this point, the stimulus was continued, but the frequency of stimulation was reduced.

Identification of recording sites

On completion of the experiment, an electrolytic lesion was made at the recording site by applying an anodal current of 10–15 μA for ≈15 s. The animal was then deeply anesthetized with urethan and perfused transcardially with normal saline followed by 10% Formalin (≈200 ml of each). Brains were postfixed in 10% Formalin for ≈2 days, cut in 100-μm transverse sections with a freezing microtome, and stained with cresyl violet. The position of the recording site was confirmed by coordinating depth measurements and track locations with histologically recovered lesions. Data from individual experiments were pooled on thalamic transverse sections on the basis of the atlas of Paxinos and Watson (1986).

Data analysis

For each unit, the mean spontaneous firing rate was calculated over the 20-s interval immediately preceding each cutaneous stimulation. Responses to cutaneous stimuli were considered significant if two or more consecutive 1-s bins in the event histogram exceeded the prestimulus mean firing rate by >2 SD. The mean spontaneous firing rates are depicted as solid horizontal lines on the event histograms; the 2-SD cutoffs are depicted as dashed lines.

All blood pressure data are presented as changes in mean arterial pressure (MAP) calculated from the following equation

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\text{MAP} = \text{systolic blood pressure} + \frac{1}{3} \times \text{pulse pressure}
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Because the focus of the current experiment is on responses evoked by cutaneous stimuli, the change in MAP or HR has been reported relative to the immediate prestimulus control (20 s before the stimulus, not pre-STR). More precisely, the maximum HR or MAP observed in the 20 s before stimulus application was subtracted from the maximum value observed during stimulus application. The peak/trough of these difference values was then determined for individual animals before and after i.t. STR. The mean of these peak/trough values (before and after STR) was compared with the use of a paired t-test and is presented in Fig. 6.

RESULTS

The effects of i.t. STR were determined on 20 medial thalamic units in 19 animals. Only one unit was studied in each animal, with the exception of one experiment in which two units were recorded simultaneously and later discriminated off-line. All of the units in this study were classified as NS because they were excited by noxious pinch applied to cutaneous sites, but not by innocuous tactile stimuli. The response properties of all the units tested were affected by i.t. STR.
Effects of i.t. STR on neuronal properties

After i.t. STR, all (formerly NS) units exhibited significant responses to innocuous tactile stimuli (brush or air jet) applied to lumbar or sacral dermatomes. The response properties of a typical NS unit in medial thalamus are outlined in Fig. 2. This unit responded exclusively to noxious pinch applied to either the tail or contralateral hind paw. innocuous brush or air jet had no effect on HR, blood pressure, or unit firing rate. In contrast, 2 min after 40 μg i.t. STR, similar brush or air jet stimuli evoked a pressor response, tachycardia, motor withdrawal reflexes, and a significant increase in the firing rate of the cell. An example of a response to air jet stimulation is shown in Fig. 2E; the novel RF for brush stimulation is depicted in Fig. 2F. Note that after i.t. STR, the air jet stimulus evoked a prolonged unit response with an afterdischarge (Fig. 2E), similar to the response observed with hind paw pinch (Fig. 2A).

Figure 3 illustrates a medial thalamic unit that exhibited phasic responses to noxious stimuli. Like the unit described in Fig. 2, this unit responded only to noxious pinch applied to either the hindlimbs or flanks. However, the response to pinch was brief and did not last throughout the stimulus application. Before i.t. STR, neither brush stimulation applied to the hindquarters nor air jet applied to the hind leg elicited any change in HR, blood pressure, or unit firing rate. In contrast, within 5 min after 40 μg i.t. STR, similar air jet or brush stimuli evoked a pressor response, tachycardia, motor withdrawal reflexes, and a significant increase in the firing rate of the cell. Note that an air jet applied to the hairy skin of the hind leg 12 min after i.t. STR evoked an abrupt phasic increase in the unit firing rate (Fig. 3B) similar to that seen with hind paw pinch before STR (Fig. 3A). After i.t. STR, brush stimulation also elicited significant unit responses, which were most pronounced at the stimulus onset, as the stimulus was applied sequentially to different regions of the body. The brush stimulus was effective in evoking unit responses at all sites tested on the trunk and hindquarters as from 2 to 2.4 mm caudal to bregma have been included on bregma –2.3 section; those between 2.4 and 2.8 mm caudal to bregma appear on bregma –2.56 section. Note that 2 units were recorded from single site in anteromedial (AM) nucleus. CL, centrolateral; CM, centromedial; GP, globus pallidus; MDC, mediodorsal central; MDL, mediodorsal lateral; MDM, mediodorsal medial; PC, paracentral; Re, reuniens; Rh, rhomboid; Rt, reticular; Sm, submedius; VM, ventromedial; VL, ventrolateral; VPM, ventroposteromedial; VPL, ventroposterolateral; mt, mammillothalamic tract.

Figure 1 illustrates the locations of the thalamic recording sites. These included four sites in the mediodorsal medial nucleus; two each in the mediodorsal, anteromedial, centromedial, reuniens, and rhomboid nuclei; and one each in the mediodorsal central, mediodorsal lateral, and submedius nuclei. Note that two units depicted in mediodorsal medial nucleus in Fig. 1 were actually in the more rostral portion of the nucleus where the division between mediodorsal medial and mediodorsal central nuclei is not sharply delimited. Also, two units were recorded from the same site in the anteromedial nucleus. The exact recording sites for three units could not be determined with certainty from the histology, but the electrode tracks and stereotaxic coordinates indicate that they were in the mediodorsal, centromedial, or submedius region of medial thalamus.

Figure 3 illustrates the relationship between cutaneous sites responsive to pinch before i.t. STR and the brush RF after i.t. STR. Because of the tendency of some medial thalamic cells to exhibit prolonged responses to repeated noxious stimuli, the pinch RF was usually not mapped completely. On the basis of the available data, the RFs for brush stimulation after i.t. STR were substantially different from the pre-STR pinch RFs. In 15 cases (Fig. 4, A–O) the brush RF was smaller relative to the pinch RF in that it failed to include all pinch sites. However, for six of these units (Fig. 4, F and K–O) the new brush RFs included sites that did not respond to pinch before i.t. STR, and in another six (Fig.
FIG. 2. Example of effects of intrathecal (i.t.) strychnine (STR) on NS neuron in medial thalamus. Unit exhibited significant increase in firing rate in response to pinch of contralateral hind paw or tail (C, filled cross), but pinching of ipsilateral hind paw (C, open cross) failed to elicit significant unit response (A). Noxious pinch also elicited blood pressure changes and tachycardia (A). Before i.t. STR, application of innocuous brush or air jet stimuli to rat hindquarters failed to alter heart rate, blood pressure, or unit firing pattern (C and D). In contrast, after 40 µg i.t. STR, similar air jet (E) or brush (F) stimuli evoked pressor response, tachycardia, motor withdrawal reflexes, and significant increase in firing rate of cell. Note afterdischarge following air jet stimulus (E). Receptive field (RF) for brush-evoked unit responses is shown in F. Horizontal lines in event histograms: mean prestimulus firing rate; dashed lines: level of 2 SD above mean. Location of recording site in anteromedial thalamic nucleus is shown on schematic diagram of rat brain in cross section (B).

4, A, B, and G–J) the brush RF did not include any sites where pinch had been applied. In two cases the brush RF was larger than the pinch RF (Fig. 4, P and Q), whereas in the remaining three cases the brush RF was comparable with the pinch RF (Fig. 4, K–T). It must be acknowledged that the RFs for brush are only approximations because, in many cases, the duration of this effect of STR was short, and the borders of the RF usually changed with time.

The cutaneous regions eliciting the most pronounced unit responses to brush were ipsilateral to the i.t. catheter (as determined by the lidocaine test) regardless of whether these regions were ipsilateral or contralateral to the thalamic recording site. This relationship is clearly demonstrated by those animals whose i.t. catheters were ipsilateral to the recording site (Fig. 4, A, F, K, and P). Although two of the units with thalamic recording sites ipsilateral to the i.t. catheter (A and P) had bilaterally symmetrical brush RFs, one of these (Figs. 2, E and F, and 4A) responded to air jet at an additional site ipsilateral to the i.t. catheter, and the other exhibited more robust responses to brushing applied ipsilateral to the i.t. catheter (Figs. 3B and 4P).

The majority of units (11 of 20) exhibited an increase in spontaneous firing rate of ≥2 times the mean rate before i.t. STR. Brush-evoked inhibition could be demonstrated in four cells with spontaneous firing rates that were high enough to provide a baseline for comparison: two with high spontaneous firing rates before STR and two that developed elevated spontaneous firing after i.t. STR. Brush-evoked inhibition after i.t. STR was transient, but when it was present the unit could not be excited from any cutaneous site. The majority of units (11 of 20) exhibited an increase in spontaneous firing rate of ≥2 times the mean rate before i.t. STR. Brush-evoked inhibition could be demonstrated in four cells with spontaneous firing rates that were high enough to provide a baseline for comparison: two with high spontaneous firing rates before STR and two that developed elevated spontaneous firing after i.t. STR. Brush-evoked inhibition after i.t. STR was transient, but when it was present the unit could not be excited from any cutaneous site. Figure 5 illustrates one of the more complicated patterns of unit responses observed in this study (see also Fig. 4L). This unit exhibited fluctuations in spontaneous firing rate that appeared to influence its responsiveness to cutaneous stimuli. At 3 min post-STR this unit exhibited a high spontaneous firing rate and no clear response to brushing, although brushing evoked a motor withdrawal reflex (not shown). By 10 min post-STR, the spontaneous firing rate was somewhat lower, and brushing the trunk, flank, and hindlimb contralateral to the recording site evoked a significant unit response (Fig. 5D). Note that the RF for brush stimulation included sites that had not responded to any type of cutaneous stimulation before STR (Figs. 5A or 4L) and was larger than the pre-STR pinch RF (note also that the RFs in Fig. 5 are mirror images of the standardized drawing in Fig. 4L, which shows the RF contralateral to the recording site on the right side of the drawing and amalgamates data from before and after i.t. STR). Two minutes after the brush-evoked unit...
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FIG. 3. Example of effects of i.t. STR on medial thalamic unit. Before i.t. STR, innocuous brush or air jet stimuli applied to rat hindquarters or trunk failed to alter heart rate, blood pressure, or unit firing rate (A). After 40 µg i.t. STR (B), similar brush stimulation evoked pressor response, tachycardia, motor withdrawal reflexes, and significant increase in unit firing rate at all sites tested (shading on rat drawing); effects of brushing more rostral sites were not tested. Cardiovascular and motor responses evoked by air jet (not shown) were less dramatic than those evoked by brushing, but unit response (B) was comparable with that evoked by pinch before i.t. STR (A). Recovery from effects of i.t. STR was evident by 22 min after drug injection (C). Solid horizontal lines: mean firing rate of unit before stimulus application. Dashed lines: 2 SD from mean. Location of recording site in mediodorsal central thalamic nucleus is shown as filled circle on schematic diagram of rat brain in cross section (D). Abbreviations as in Fig. 1.

response, hind paw pinch also evoked an abrupt increase in firing rate (Fig. 5D) and an afterdischarge more substantial than that associated with pinch stimulation before STR (Fig. 5A). A few minutes later, the unit again developed a high spontaneous firing rate and responses to brush or pinch could no longer be evoked (Fig. 5E). Cardiovascular parameters were not monitored in this animal, but brush stimulation evoked motor withdrawal reflexes at 3, 6, 10, and 16 min post-STR.

Although not the focus of the study, the duration of STR-dependent, brush-evoked unit responses could be estimated for 16 of the 20 units studied. The remaining units were lost before recovery from the effects of STR was observed. Five units exhibited extremely brief responses to brush, which were detected only once during the post-STR period; no unit response to brush was evoked by a second stimulus applied <5 min after the first significant brush-evoked unit response. At the other extreme, one unit exhibited a prolonged responsiveness to brush, which persisted for ~2 h after STR injection and outlasted all overt signs of allodynia; no brush-evoked cardiovascular or motor responses were observed in this animal beyond 24 min post-STR. The onset of symptoms of STR-dependent allodynia (next section) coincided with the onset of brush-evoked unit responses for 9 of the 20 units studied. In nine of the remaining cases, allodynia was observed before the onset of brush-evoked unit responses, and in one instance, allodynia was observed after the onset of the unit response. Allodynia was not observed in one animal.

STR-dependent allodynia

Evidence of STR-dependent allodynia, in the form of brush-evoked cardiovascular or motor responses, was observed in 18 of the 19 rats. Blood pressure and HR were monitored in 14 of the 18 animals, but 1 animal that received a higher dose of i.t. STR (50 µg) was omitted from the mean data. In the control period, before administration of i.t. STR, virtually no changes in MAP or HR were observed as a result of brush stimulation (Fig. 6). However, after i.t. STR, a similar brush stimulation evoked tachycardia and significant changes in MAP. Evoked changes in HR followed a consistent pattern with each stimulation; tachycardia appeared abruptly the first time a sensitive dermatome was stimulated and persisted beyond the termination of the stimulus (e.g., Figs. 2 and 3). Changes in MAP were more vari-
able. They were often biphasic, with an initial fall and subsequent rise, but in some instances only a fall or rise was observed. The maximum changes in HR and MAP evoked by brush stimulation are shown in Fig. 6.

The cardiovascular effects of STR appear to be related to cutaneous stimulation rather than a direct effect on sympathetic efferents, because they were abrupt and temporally related to the stimulus (e.g., Fig. 2E). Furthermore, the average basal HR and MAP were unaffected by i.t. STR. The mean prestimulus MAP (104.3 ± 4.9 mmHg, mean ± SE; n = 13) just before the greatest brush-evoked increase in MAP did not differ significantly from the average peak MAP before i.t. STR (108.3 ± 5.1 mmHg). Similarly, the prestimulus HR (417 ± 11 bpm) immediately preceding the maximum brush-evoked increase in HR did not differ significantly from the mean HR in the absence of STR (421 ± 11 bpm).

Brush-evoked motor reflexes were observed during at least one stimulation in 14 animals. Motor responses were abrupt and were time linked to the first application of the stimulus to a sensitive cutaneous region. Repeated stimulation at the same site resulted in adaptation, with subsequent stimuli eliciting either no motor withdrawal or a much less vigorous response.

**DISCUSSION**

STR-dependent allodynia is associated with changes in the responses of medial thalamic neurons

The present study is the first to demonstrate changes in nociceptive neurons concurrently with overt signs of i.t.-STR-dependent allodynia. All of the medial thalamic units examined in the current experiments could only be activated by nociceptive stimuli, except immediately after i.t. STR, when they all developed responsiveness to low-threshold brush or air jet stimulation. Because there is strong evidence that the direct receptor-mediated actions of i.t. STR occur locally at the spinal level (see below), the current results indicate that STR-induced changes at the spinal level give rise to major changes in neuronal properties at the level of the medial thalamus and therefore presumably also at the cortical level. Thus i.t. STR does not simply modify the afferent (or efferent) components of spinal reflex arcs, but also results in altered responses of medial thalamic neurons. This study also shows that spinal application of STR at doses and by the route of administration known to produce behavioral indexes of allodynia produces major changes in the spinal neurons that give rise to ascending pathways influencing medial thalamus. These data provide support for the growing realization of the importance of glycine in the control of nociceptive transmission and for disruption of the normal functioning of this system as a mechanism underlying the development of some types of neuropathic pain. Recent studies have also implicated g-aminobutyric acid as another important spinal inhibitory transmitter involved in the modulation of nociceptive transmission (Lin et al. 1994; Peng et al. 1996).

Evidence for a spinal site of action of i.t. STR

Behavioral observations indicate that the STR-induced disinhibition observed at the spinal level is not generalized

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**FIG. 4.** Changes in sensory modality and RFs of NS neurons in rat medial thalamus. Filled crosses: sites where pinch elicited significant unit response. Open crosses: sites where pinch failed to elicit unit response. After i.t. STR, all of these (formerly NS) neurons were excited by brushing over cutaneous regions indicated by shading. (Note that in some cases shading used to delimit brush RFs is obscured by crosses used to mark pinch stimulation sites. In these cases brush RF was continuous even though shading is interrupted by crosses). Cutaneous RFs contralateral to thalamic recording site are shown on right side of drawings for ease of comparison among drawings (regardless of whether recordings are from left or right thalamus). Position of i.t. catheter in lumbar subarachnoid space (as determined by lidocaine test) was contralateral to thalamic recording site in all but 4 animals (A, F, K, and P). Lidocaine effect was bilateral in 1 animal (E), indicating that i.t. catheter was near midline of spinal cord. Dose of i.t. STR was 40 μg except in cases K and N, which received 60 and 50 μg, respectively. Unit numbers and thalamic recording sites are indicated below respective drawings. Abbreviations as in Fig. 1. Recording site was unknown (Unk) for 3 units.
to the entire CNS, but rather is a specific effect of STR on spinal segments near the i.t. drug delivery site. The most pronounced unit responses to brush in the current study were evoked from cutaneous sites ipsilateral to the i.t. catheter (as determined by the lidocaine test) regardless of whether these regions were ipsilateral or contralateral to the thalamic recording site. In accordance with these observations, a previous study (Sherman and Loomis 1995) demonstrated that cutaneous regions with abnormal tactile sensitivity, as determined by the ability of hair deflection to evoke motor withdrawal reflexes, were also found ipsilateral to the site of i.t. STR delivery. Temporal changes in cutaneous sensitivity were consistent with a caudal spread of STR in the cerebrospinal fluid following i.t. injection. The abrupt onset of STR-dependent allodynia, and the failure of similar doses of intravenous STR (40–200 μg) to elicit allodynia (Sherman and Loomis 1994), provide further evidence for a specific segmental effect of STR on the spinal cord rather than a more global action on the entire CNS.

**Mechanisms underlying alterations of medial thalamic neuron responses**

Because the direct actions of i.t. STR occur at the spinal level (see above), the changes observed in medial thalamus must be due to alterations in the ascending inputs to these neurons. All of the neurons in our study were of the NS type, suggesting that they received either direct or indirect inputs from NS spinal neurons. However, it is also possible that these thalamic NS neurons received inputs originating from WDR spinal neurons, or even possibly from some low-threshold mechanoreceptive neurons, but that these low-threshold afferent inputs were not powerful enough to produce changes in firing rate. If i.t. STR caused spinal NS neurons to start responding to low-threshold inputs and/or...
caused increased firing of WDR and low-threshold mechanoreceptive neurons to low-threshold tactile inputs, this would explain the lowering in threshold of the NS neurons observed in our study. There are indeed data from spinal cord studies suggesting that STR can produce these types of changes in spinal dorsal horn neurons. For example, previous studies have demonstrated that NS units have subliminal input from low-threshold afferents and that these can be unmasked by STR (Woolf and King 1990). In the cat trigeminal nucleus caudalis, locally infused STR enhanced responses of WDR neurons to both innocuous tap and noxious dental pulp stimulation (Khayyat et al. 1975). In the cat spinal cord, Sorkin and Puig (1996) recently confirmed the ability of repetitive natural innocuous brush stimulation to cause windup of some rat WDR dorsal horn neurons following iontophoretic STR. Windup usually only occurs with peripheral stimuli that are strong enough to activate nociceptive afferents, and therefore these findings suggest that STR is increasing the effectiveness of low-threshold inputs to WDR cells.

Alldynia

The onset, duration, and quality of alldynia observed in the present experiments were consistent with previous reports of this phenomenon (Sherman and Loomis 1994–1996; Yaksh 1989). These experiments were conducted under conditions virtually identical to those of the Sherman and Loomis studies (cited above), facilitating comparison of results between these studies. However, the maximum changes in HR and MAP evoked by brush stimulation were less pronounced in the current study than in earlier reports. For example, Sherman and Loomis (1994) reported maximum evoked increases in HR and MAP of 29 ± 1.1 (SE) bpm and 15 ± 1.0 (SE) mmHg, respectively, when hair deflection was applied to the hindquarters after 40 µg i.t. STR (n = 28). In the present study, the peak brush-evoked increases in HR and MAP were 12.5 ± 2.8 bpm and 8.8 ± 1.6 mmHg (n = 13). These differences may be explained by differences in technique, because the hair deflection stimulus in the Sherman and Loomis (1994) study was applied to the most sensitive site on the hindquarters for 2 min, whereas in the current study the brush RF was mapped by applying stimuli at different sites for only a few seconds. The reduced magnitude of cardiovascular responses was thus a predictable outcome of decreasing the stimulation time (which was necessary for the objectives of the current study) and does not detract from the validity of the results.

The onset of STR-dependent alldynia coincided with the onset of brush-evoked unit responses for 9 of the 20 units studied. The lack of exact temporal correlation between the onset of unit changes and alldynia in the remaining cases does not necessarily indicate that medial thalamus had no role in the manifestation of alldynic symptoms. Because a
large population of neurons is required to elicit gross motor and cardiovascular responses, the particular unit monitored in some experiments might not have been recruited for the overall population response to STR. The critical observation is that all of the medial thalamic units investigated in the present study exhibited dramatic functional changes within minutes of i.t. STR administration, and these changes occurred during the time course of STR-dependent allodynia. Thus these observations are consistent with the involvement of medial thalamic neurons in the manifestation of STR-dependent allodynia.

Conclusions

In the current study we investigated the effects of i.t. STR on the response properties of NS neurons in medial thalamus. After i.t. STR, neurons that were initially NS became responsive to brush stimulation. The ability of NS cells in medial thalamus to respond to tactile input after i.t. STR indicates that the direct actions of STR on spinal neurons are reflected supraspinally by changes in nociceptive processing in medial thalamus. These data provide strong support for recent studies suggesting that low-threshold inputs can produce pain via central mechanisms under some abnormal conditions and also suggest that medial thalamic structures may play a role in mediating STR-dependent allodynia. Further studies are required to determine the types of spinal neurons involved in relaying abnormal sensory input to medial thalamus and the specific ascending sensory pathway(s) involved.

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