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# A possible interface between autonomic function and pain control: opioid analgesia and the nucleus tractus solitarius

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Opioid peptides appear to be important neurochemical mediators in central nervous system mechanisms of analgesia, cardiovascular control, and many endocrinological responses to stress. The nucleus tractus solitarius (NTS), a brain region expressing all 3 opioid peptide families, is also associated with regulation of autonomic and endocrine functions. We now report that electrical stimulation of the NTS causes pronounced analgesia in rats. This analgesia appears to involve opioids and is pharmacologically dissociable from the hemodynamic changes elicited by NTS stimulation. These results suggest the NTS as a neural substrate for inter-relationships between stress, cardiovascular function, alterations in respiration, and pain sensitivity.

## INTRODUCTION

Intimate associations between pain sensitivity, cardiovascular function, opioid peptides, and the response to stress are now beginning to be revealed<sup>10, 28</sup>. The nucleus tractus solitarius (NTS), located in the caudal medulla and well-known to be involved in cardiovascular and respiratory regulation (e.g. ref. 8) may be a neural locus for these interactions. Converging lines of evidence have led us to hypothesize that the NTS could also be an integral part of endogenous pain-inhibitory systems that involve opioid peptides. Biochemically, this region contains opioid receptors and peptides derived from all 3 opioid precursor families: pro-opiomelanocortin, pro-enkephalin, and pro-dynorphin<sup>2,11,12,18,32</sup>. Anatomically, the NTS has extensive projections to, or receives afferents from, several brain loci thought to be involved in pain and pain-inhibition (e.g., periaqueductal/periventricular gray matter, nucleus raphe magnus, and the spinal cord)<sup>23,29</sup>. Behaviorally, microinjection of morphine<sup>24</sup> or noradrenergic agents<sup>21</sup> into the NTS causes analgesia.

We now report that electrical stimulation of the NTS causes opioid-mediated analgesia in pentobarbital-anesthetized rats. Thus, while the NTS is not classically thought of as being along the pain pathway, our findings suggest the existence of a close structural and functional interface between cardiovascular/respiratory control and regulation of responsiveness to noxious stimuli. This interface appears to take place at a very early autonomic relay station in the central nervous system. A preliminary report of these data has been made<sup>13</sup>.

## MATERIALS AND METHODS

### *Experiment 1. Analgesic effect of NTS stimulation*

Subjects were male Sprague–Dawley rats (200–250 g; Charles River Laboratories, Worcester, MA). Pain sensitivity was assessed using the tail-flick test<sup>7</sup> with a maximum response latency of 10 s imposed to minimize tissue damage. In the present experiments, the analgesic effect of brain stimulation was determined in rats anesthetized with sodium pentobarbital. That behavioral measurements ob-

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tained in anesthetized animals reflect responses in the awake state is supported by the fact that tail-flick latencies in the anesthetized rats are similar, or slightly decreased, compared to those exhibited by awake rats and by findings that stimuli which modify tail-flick latencies (e.g. electrical brain stimulation, footshock, morphine) cause similar behavioral changes in awake and anesthetized rats<sup>22,31,34,35,44</sup>.

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and mounted in a stereotaxic frame. The surface of the brainstem at the level of the obex was exposed and the stimulating electrode (a twisted pair of nichrome enamel insulated wires 0.125 mm diameter) lowered approximately 1.0–1.5 mm into the brain. The electrical stimulus was produced using a Grass model S88 stimulator and consisted of monopolar, biphasic square-wave pulses (50  $\mu$ s pulses separated by a 100- $\mu$ s interval) delivered at a rate of 20 pulses/s and ranging in intensity from 125 to 750  $\mu$ A. (Note: caution must be exercised in direct comparison of brain stimulation threshold values obtained by various researchers. While these intensities of stimulation used herein may appear high, the pulse duration is very brief.)

Prior to brain stimulation, baseline tail-flick latencies were determined at 1-min intervals until a stable response was obtained. Electrical stimulation, beginning with an intensity of 125  $\mu$ A was then delivered for 30 s. Tail-flick testing resumed immediately upon termination of the stimulation and continued with trials at 1-min intervals until tail-flick latencies returned to baseline levels. If analgesia was not observed (i.e. poststimulation response latency was less than 1.5 times the baseline latency), the stimulation current was increased by 125  $\mu$ A, and this procedure was repeated. If no analgesia was obtained at the maximal stimulation intensity of 750  $\mu$ A, the electrode was repositioned and testing begun again. At some electrode placements excessive motor stimulation (e.g. twitching of the limbs, arching of the back) or cessation of respiration were observed; when these changes occurred the electrode was repositioned. During the behavioral testing procedures, animals were given additional anesthetic (10.0 mg/kg, i.p.) as needed.

When stimulation at a particular site was found to cause an increase in tail-flick latency, pharmacological characterization to this analgesia was begun. Ani-

mals were treated according to one of two protocols. After determination on the threshold current for eliciting analgesia, some rats ( $n = 6$ ) were injected with physiological saline (1 ml/kg, s.c.). After 10 min, baseline tail-flick latencies were determined and the threshold stimulation current previously found to elicit analgesia was applied. If this stimulus still caused elevations in tail-flick latencies, the animals were then injected with the opiate antagonist, naloxone (10 mg/kg, s.c.), and the testing procedure repeated 10 min later. To control for factors that may have affected the behavior of this group of rats, such as duration of anesthesia or possible effects of repeated brain stimulation, a second group of rats ( $n = 6$ ) was tested in a similar manner but without the saline injection.

To determine the anatomical specificity of the sites supporting stimulation-produced analgesia, several electrode penetrations (2–4/rat) were made in the region of the NTS in control rats ( $n = 6$ ). In these animals a maximal stimulus (750  $\mu$ A) was applied and alterations in tail-flick latencies noted. Although the purpose of the group was to demonstrate that stimulation of structures adjacent to the NTS does not cause analgesia, a stimulation site that did cause increases in tail-flick latencies was located in each rat, attesting to the viability of each preparation.

At the end of each experimental session, the stimulation site(s) was marked with an electrolytic lesion, the animals perfused intracardially with a saline/formalin solution, and the brain prepared with Nissl stains for histological verification of the electrode site. Location of the electrode was evaluated using the atlas of Paxinos and Watson<sup>26</sup>. Behavioral data were analyzed with Student's *t*-test for dependent samples.

#### *Experiment 2. Relationship between NTS stimulation-produced analgesia and hemodynamic changes*

To assess the possible relationship between NTS stimulation-produced analgesia and hemodynamic changes, we examined simultaneously the effects of NTS stimulation on pain sensitivity and arterial blood pressure. Rats ( $n = 8$ ) were prepared for brain stimulation as before and the femoral artery catheterized for measurement of blood pressure. Blood pressure was recorded using a Statham Laboratories pressure transducer (Model P23AC) and a Grass polygraph.

Brain stimulation and behavioral testing procedures were carried out as before.

Observations from each stimulation site were classified non-parametrically on two dimensions: analgesia vs no analgesia, and blood pressure increased, decreased, or did not change. To test for a possible association between blood pressure change and analgesia, these data were analyzed statistically using a Chi-square test.

Finally, in some of the rats catheterized for blood pressure measurements, further dissociation of the analgesic and hemodynamic effects of NTS stimulation was accomplished pharmacologically. In each rat, a stimulation site was located that elicited increases in tail-flick latency accompanied by hypertension (the most common effect of NTS stimulation). Rats ( $n = 3$ ) were then given either trimethaphan camsylate (Arfonad; Roche Laboratories, Nutley NJ; 4 mg/kg, i.v.), a peripherally acting antagonist of sympathetic nervous system activity, or naloxone (10 mg/kg;  $n = 3$ ). Testing resumed 10–20 min later and the effects on blood pressure and pain sensitivity of threshold stimulation observed.

## RESULTS

Stimulation of those sites in or near NTS (Fig. 1 top,  $n = 12$ ) was found to elicit marked elevations in tail-flick latencies, whereas stimulation of placements dorsal, lateral, or ventral to this nucleus (Fig. 1 top,  $n = 12$ ) was without effect. Following threshold stimulation of NTS sites, tail-flick latencies were significantly elevated compared to baseline values ( $P < 0.01$ ; Fig. 1 bottom, A,B). The duration of stimulation-produced analgesia was variable across subjects. Defining analgesia as an increase in tail-flick latency of at least 1.5 times the baseline value, poststimulation analgesia lasted between 1 and 9 min. Neither baseline nor poststimulation latencies were significantly altered following saline injections (Fig. 1 bottom, B). Naloxone administration, by contrast, significantly attenuated the analgesic response to threshold stimulation ( $P < 0.01$ , compared to either postsaline or threshold latencies; Fig. 1 bottom, A,B).

By varying stimulation site and intensity, a total of 167 measurements of poststimulation blood pressure and pain sensitivity were obtained from the 8 rats

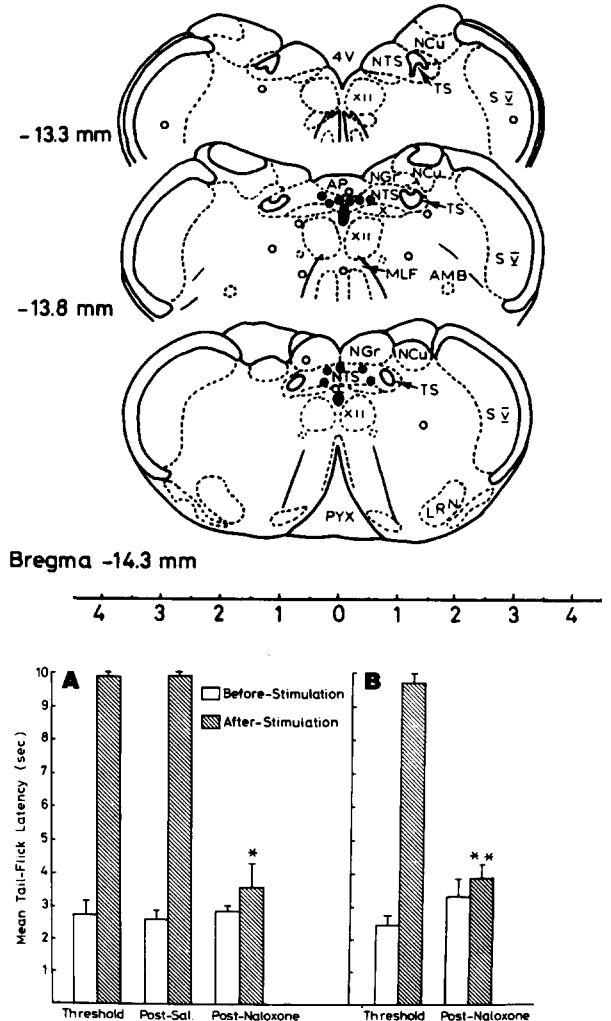


Fig. 1. Top: location of stimulation sites. Sites that supported analgesia are depicted by closed circles ( $n = 12$ ) and ineffective sites are indicated by open circles ( $n = 12$ ). Bottom: effect of NTS stimulation on tail-flick latency. Panels A and B show data from rats tested for pain sensitivity before and after NTS stimulation. NTS stimulation markedly increased tail-flick latency compared to baseline values (A and B). Injection of saline did not affect NTS stimulation-produced analgesia (B). Naloxone, markedly attenuated stimulation-produced analgesia (A, \* differed from both postthreshold and postsaline latencies,  $P < 0.01$ ; B, \*\* differed from postthreshold latency,  $P < 0.01$ ). AMB, ambiguous nucleus; 4V, fourth ventricle; LRN, lateral reticular nucleus; NTS, nucleus tractus solitarius; NCu, nucleus cuneatus; PYX, decussation of the pyramidal tract; S V, spinal trigeminal nucleus; TS, tractus solitarius; X, dorsal motor nucleus of vagus; XII, hypoglossal nucleus.

tested. These data are presented in Table I. Statistical analysis indicated no significant association between changes in blood pressure and analgesia (Chi square = 1.38,  $P > 0.05$ ). Furthermore, blockade of

TABLE I

*Classification of the effects of electrical stimulation in and around the NTS on arterial blood pressure and pain sensitivity*

These data summarize the effects of 167 stimulation sites. There was no significant association between elicitation of analgesia and alterations in blood pressure (Chi square = 1.38,  $P > 0.05$ ).

	Arterial blood pressure		
	Decrease	No change	Increase
Analgesia	14	11	45
No analgesia	26	17	54

sympathetic activity with Arfonad eliminated the pressor response without affecting analgesia. At similar stimulation sites in other rats, treatment with naloxone blocked stimulation-produced analgesia without markedly affecting the hypertension.

#### DISCUSSION

Taken together, these findings demonstrate that electrical stimulation of the nucleus tractus solitarius causes analgesia that is prevented by naloxone suggesting mediation by opioid peptides. That control stimulation sites, near the NTS, were ineffective in causing analgesia indicates that the present results are not due to stimulation of adjacent structures, many of which have been implicated in mechanisms of pain-inhibition (e.g. the dorsolateral funiculus<sup>4</sup> or the dorsal columns<sup>33</sup>). Comparison of stimulation-produced analgesia elicited from the NTS to those forms previously reported following stimulation of more classical pain-inhibitory loci<sup>3,6,20,25</sup> suggests that NTS-derived analgesia is comparable in magnitude with these other forms of brain stimulation-produced analgesia, but that it is typically of longer duration and is more sensitive to antagonism by naloxone. Perhaps the latter finding indicates the production of a more 'pure' opioid analgesia.

That the pain regulatory and cardiovascular mechanisms can be dissociated pharmacologically or by manipulating the stimulation site, provides evidence that these responses are subserved by separable neural elements and that increases in tail-flick latency are not secondary to alterations in blood pressure, per se. This dissociation between analgesia and cardiovascular changes is congruent with previous re-

ports indicating the independence of opioid-mediated decreases in pain sensitivity and hypertension<sup>30,42,43</sup>. These results, however, do not imply that antinociception and blood pressure are independent events in response to natural stimuli. Moreover, since analgesia has been associated with increases and decreases in blood pressure, it may be that the critical signal for stimulation of opioid analgesia systems is deviation from homeostasis. It should be noted that another effect of NTS stimulation, alteration in respiration, may be linked to changes in pain-sensitivity. The present study, however, did not examine this variable systematically. Nevertheless, the fact that blood pressure control, respiratory regulation, and antinociception share common anatomical, and possibly biochemical, substrates may begin to reveal a neural basis for reported changes in pain sensitivity accompanying stress, cardiovascular hypertension<sup>8,10,28</sup>, and decreased pain-responsiveness induced by alterations in respiratory pattern, such as LaMaze analgesia<sup>40</sup>.

Current conceptions of the organization of an endogenous opioid analgesia system could be significantly affected by integration of the NTS. In general, this analgesia system has been described as arising in the periventricular/periaqueductal gray of the medial brainstem, involving monoaminergic neurons of the nucleus raphe magnus and the nucleus reticularis paragigantocellularis, descending to the spinal cord via the dorsolateral funiculus, and exerting pain-inhibition in the spinal dorsal horn (for reviews see refs. 3, 15, 39). While this well-defined opioid analgesia system can be activated by direct electrical stimulation or administration of opiate drugs<sup>3,15,20,25,36,37,39,41</sup>, the origin of the neural signals capable of energizing this system in response to environmental stimulation is not known. The NTS is a major recipient of somato/visceral sensory information and anatomically connected to pain/inhibitory regions making it a likely candidate for such a role.

Finally, it may be that the NTS, by providing a direct input to endogenous analgesia systems, serves as a crucial interface between environmental stressors and neural substrates of pain inhibition. For example, in man and animals, exposure to stress appears to be an adequate stimulus for activation of endogenous opioid pain-inhibitory systems (e.g. refs. 1, 5, 16, 38). One form of opioid-mediated, stress-induced

analgesia<sup>1,14</sup> can be attenuated by unilateral vagotomy<sup>19</sup> or adrenal demedullation<sup>17</sup>, indicating the importance of peripheral autonomic systems in this response. Thus, since exposure to stress causes analgesia and is accompanied by numerous autonomic sequelae, and because the NTS is known to be involved in these autonomic responses and NTS stimulation elicits pain-inhibition, it is reasonable to hypothesize that an important linkage between stressful stimuli and endogenous analgesia systems occurs via the nu-

cleus tractus solitarius.

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