Stimulation of the hypothalamic paraventricular nucleus produces analgesia not mediated by vasopressin or endogenous opioids

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The analgesic effect of electrical stimulation of the hypothalamic paraventricular nucleus (PVN) was studied. Additionally, the involvement of vasopressin and opioid peptides in this process was examined by comparing vasopressin-deficient (Brattleboro) and Long–Evans rats and by administering the opiate antagonist naloxone. Rats were chronically implanted with a stimulating electrode in the parvocellular (PVN-Pc) and magnocellular (PVN-Mg) divisions of the PVN. At least 10 days after surgery, the analgesic effects of PVN stimulation were examined in lightly anesthetized rats, using the tail-flick method, and in unanesthetized rats, using the hot-plate test. PVN stimulation produced marked analgesia in both tests. Current threshold for analgesia was lower from PVN-Pc than from PVN-Mg. Threshold did not differ significantly between Brattleboro and Long–Evans rats and was not affected by naloxone administration. The results indicate that the PVN is part of the brain's pain inhibitory system, and show that the analgesia induced by PVN stimulation is not mediated by either vasopressin or opioid peptides.

INTRODUCTION

In the last two decades, many structures at various levels of the neuraxis have been implicated in the modulation of pain responsiveness. Several of these structures are located in the hypothalamus. Stimulation of lateral, medial, and posterior hypothalamic areas has been reported to produce analgesia. Several lines of evidence suggest that the paraventricular nucleus (PVN) of the hypothalamus is also involved in pain modulation. (1) Neurons within the PVN project to various central nervous system areas that are involved in nociception, including the periaqueductal gray (PAG), nucleus raphe magnus, nucleus of the solitary tract, the sensory nuclei of the trigeminal and vagus nerves, and the substantia gelatinosa of the spinal cord. PVN neurons synthesize and secrete many neuropeptides, some of which have been shown to produce analgesia upon exogenous administration, e.g., vasopressin, Met- and Leu-enkephalin, dynorphin, somatostatin, neurotensin, TRH and CRF and project to the medulla and/or spinal cord. (2) PVN neurons synthesize and secrete many neuropeptides, some of which have been shown to produce analgesia upon exogenous administration, e.g., vasopressin, Met- and Leu-enkephalin, dynorphin, somatostatin, neurotensin, thyrotropin-releasing hormone (TRH), and corticotropin-releasing factor (CRF). (3) The PVN is a major integrating area for the stress response, which is known to activate the endogenous pain inhibitory system. The finding that PVN lesions abolished the analgesic response to stress supports this conclusion.

In the first experiment of the present study, the analgesic effects of PVN stimulation were studied. Both magnocellular and parvocellular divisions of the PVN were stimulated. Previous studies showed that cells in the magnocellular division synthesize mainly vasopressin and oxytocin and project to the posterior pituitary, whereas cells in the parvocellular division synthesize these two neuropeptides as well as several others including opioids, somatostatin, neurotensin, TRH and CRF and project to the medulla and/or spinal cord. Additionally, we examined the possibility that PVN stimulation-produced analgesia (SPA) is mediated by the neuropeptide vasopressin. Vasopressin (VP) and several of its analogues were found to produce marked analgesia when injected either systemically, intracerebroventricularly, or intrathecally, but see ref. 36. Genetic and experimental manipulations in endogenous VP levels were also found to modify responsiveness to painful stimuli. The possibility that vasopressinergic projections from the PVN are involved in pain modulation was examined by comparing PVN SPA in Brattleboro (DI) rats (which are homozygous for diabetes insipidus and lack the ability to synthesize VP) and Long–Evans (LE) rats (the strain

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from which DI rats were derived). In a second experiment, we examined the effects of the opiate antagonist naloxone on PVN SPA in LE rats. Neurons within the PVN have previously been shown to synthesize enkephalins and dynorphin\cite{31}, and projections of these cells to the brainstem and spinal cord have been reported\cite{15,18,45}. Additionally, projections from the PVN have been implicated in regulating \( \beta \)-endorphin release from the pituitary during stress\cite{35}.

**MATERIALS AND METHODS**

**Subjects**

Subjects were male rats (250–400 g) of the LE and DI strains (Blue Spruce Farms, Altamont, NY). Rats were housed individually in standard stainless-steel cages with free access to food and water. Experimental manipulations were conducted in the first half of the dark phase of a 12-hr light-dark cycle.

**Surgery**

Subjects were implanted with a single stimulating electrode under pentobarbital anesthesia (50 mg/kg, i.p.). In the first experiment, bipolar electrodes were implanted, constructed of Teflon-coated stainless-steel wires cut to equal lengths and bored of insulation at the cross section of their tips, with a tip size of 200 \( \mu \)m. In the second experiment, monophasic electrodes were implanted, constructed from epoxy-coated Number 00 insect pins, with a tip size of 80 \( \mu \)m. Animals were placed in a stereotaxic apparatus, their skulls were exposed, and a burr hole was drilled over either the left or right PVN. The electrode was lowered into the PVN (A, -1.2; L, 0.2 (paraventricular); 0.5 (magnocellular); V, 8.3 mm). Three stainless-steel screws were inserted into the skull and the electrode and screws were covered with dental cement. Animals were given 10 days to recover from surgery.

**Procedure**

The analgesic effect of PVN stimulation was assessed using the D’Amour and Smith tail-flick method\cite{36} (Expt. 1 and first phase of Expt. 2) and by the hot-plate method (second phase of Expt. 2). For the tail-flick experiments, a ‘cut-off’ time of 7.0 s was used for automatic termination of a tail-flick trial. The analgesic threshold for PVN stimulation was defined as the minimal current intensity sufficient to elevate tail-flick latency to the cut-off value. Twenty min after injecting rats with 40 mg/kg pentobarbital, baseline responsiveness on the tail-flick test was assessed by determining the mean response latency for the last 3 of 5 trials. Stimulation was delivered at a frequency of 50 Hz and a pulse width of 400 \( \mu \)s. To determine SPA current threshold, stimulation began at an intensity of 20 \( \mu \)A and was increased in 10-\( \mu \)A steps up to 100 \( \mu \)A, and in 20-\( \mu \)A steps from 100 to 300 \( \mu \)A until a current level was reached that completely inhibited the tail-flick response. At that time, the stimulation procedure was stopped and tail-flick testing continued until the response latency was equal to or shorter than the prestimulation baseline. Brain stimulation was then delivered at the last tested intensity. If a cut-off latency was reached again, this current level was defined as the SPA threshold. However, if cut-off latency was not reached, threshold determination resumed as before until a new threshold was attained.

In the second experiment, following SPA threshold determination, half the rats were injected with naloxone (10 mg/kg in a volume of 1 ml/kg); the others with saline. Twenty min later, SPA threshold was retested by stimulating the rats at the previously determined threshold. If the current intensity was no longer sufficient to produce cut-off latency, it was increased in 10- or 20-\( \mu \)A steps. This procedure was repeated 2 days later with reversed drug assignment. Five days later, PVN SPA was assessed in the same animals, now unanesthetized, using the hot-plate method. Animals were placed on a 52 °C metal surface covered with an insulating cardboard. One min later, the cardboard was removed and the latency to lick the hind paw was measured. This procedure was repeated 15 min later. At that time, the PVN was stimulated in half the rats at their previously established current threshold, starting 10 s before removal of the cardboard and through the termination of the test.

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**Fig. 1.** A: serial sections at the level of the PVN showing localization of electrode tips in Expt. 1. Open symbols (left side) represent electrode locations in Brattleboro (DI) rats, whereas filled symbols (right side) represent electrode locations in Long–Evans (LE) rats. Circles represent SPA threshold lower than 150 \( \mu \)A, triangles SPA thresholds between 150–300 \( \mu \)A, and squares SPA thresholds higher than 300 \( \mu \)A. AHy, anterior hypothalamus; f, fornix; opt, optic tract; PaAP, paraventricular hypothalamic nucleus, anterior parvo-cellular part; PaLM, paraventricular hypothalamic nucleus, lateral magnocellular part; PaMP, paraventricular hypothalamic nucleus, medial parvo-cellular part; Pe, periventricular hypothalamic nucleus; Re, reuniens thalamic nucleus; sex, spinal optic decussation; VMH, ventromedial hypothalamic nucleus; ZI, zona incerta; 3V, third ventricle. B: localization of electrode tips in LE rats (both sides of figure) in Expt. 2. See A for details.
The remaining animals were connected to the wire but were not stimulated. PVN stimulation appeared to be aversive in 3 animals, which exhibited escape responses at stimulation onset. In these rats, the threshold current was lowered to half of the previously established value, which immediately stopped the escape attempts. To avoid tissue damage, animals that did not display hind paw lick were removed from the plate after 45 s. The entire hot-plate test procedure was repeated 2 days later with the stimulation/non-stimulation assignment reversed. A percent of the maximum possible analgesic effect (MPAE) was computed according to the formula: percent MPAE = [(TR — BL)/(45 — BL)] × 100 where TR and BL are the latencies to lick the hind paw on the second (treatment) and the first (baseline) measurement, respectively, of each session.

**Histology**

Following testing, a small lesion was produced at the stimulation site by passing direct anodal current of 1.0 mA for 2 s through the stimulating electrode. Each rat was then given an overdose of pentobarbital and intracardially perfused with 10% formalin. Brains were sectioned at 50 μm, and stained with thionine for subsequent verification of the electrode placement.

**Data analysis**

The results of the first experiment were analyzed by a two-way ANOVA with the strain (LE vs DI) and the location (PVN-Pc vs PVN-Mg) as between-subjects factors. The results of the tail-flick test in the second experiment were analyzed by two-way ANOVAs with the location (PVN-Pc or PVN-Mg) as a between-subjects factor and the drugs (saline or naloxone) and the time (before and after injection) as repeated measures. The results of the hot-plate tests were analyzed by t-tests for dependent samples.

**RESULTS**

Histological examination revealed that in the first experiment electrode tips were located in the parvocellular PVN (PVN-Pc) of 13 LE and 8 DI rats, and in the magnocellular PVN (PVN-Mg) of 6 LE and 4 DI rats. Electrode tips were located outside the PVN in 20 LE and 5 DI rats (Fig. 1A). In the second experiment electrodes were located in the PVN-Pc and PVN-Mg of 6 and 4 LE rats, respectively. Two electrodes were located outside of the PVN (Fig. 1B).

In the first experiment, PVN stimulation was found to produce marked analgesia. Stimulation of most locations outside the PVN did not produce analgesia at the highest stimulation level tested (300 μA) (Fig. 1A). PVN SPA could be observed at stimulation onset and terminated immediately after stimulation offset. Mean (± S.E.M.) threshold currents for stimulation of the PVN-Pc and PVN-Mg of LE and DI rats are presented in Fig. 2. ANOVA revealed a significant location effect (F1,27 = 22.2, P < 0.001), indicating that higher current intensities were needed to produce analgesia from PVN-Mg than from PVN-Pc. The difference in threshold between the two strains, as well as the strain by location interaction were not significant (P > 0.1).

In the second experiment, PVN stimulation once again caused pronounced analgesia in LE rats. As in the first experiment, the threshold current for analgesia was lower in PVN-Pc than in PVN-Mg (F1,8 = 8.64, P < 0.05). Overall, threshold after injection of both saline and naloxone was higher than before injection (F3,24 = 3.17,
$P < 0.05$ (Fig. 3). However, there was no difference between the elevation in threshold after saline or naloxone in either location of the PVN ($P > 0.1$).

Stimulation of the PVN was also effective in producing analgesia in awake animals using the hot-plate test (Fig. 4). The percent MPAE in both PVN-Pc and PVN-Mg was 10% in the non-stimulation session and 100% in the stimulation session ($t_s = 23.4$ for the PVN-Pc and $t_s = 29.6$ for the PVN-Mg, $P < 0.001$ for both).

**DISCUSSION**

The results indicate that stimulation of the PVN produces marked analgesia in both awake and pentobarbital-anesthetized rats. PVN stimulation inhibited spinal reflexes, indicated by suppression of the tail-flick response, as well as a more integrated behavioral response to noxious stimuli, indicated by inhibition of paw licking in the hot-plate test. The finding that stimulation of most locations outside the PVN did not produce analgesia, and the differences in SPA threshold within subdivisions of the PVN, suggest that stimulation of the PVN itself (rather than current spread to adjacent areas) produces analgesia. The possibility that at least some SPA from areas adjacent to the PVN-Pc, including PVN-Mg, resulted from current spread to PVN-Pc cannot be ruled out.

The demonstration of PVN SPA is consistent with previous studies showing involvement of hypothalamic areas in the modulation of pain responsiveness$^{19,38,42,50}$. Furthermore, this finding may indicate that SPA obtained from some or all of the previously studied hypothalamic sites located posterior to the PVN results from stimulation of descending fibers from the PVN. A similar conclusion was suggested when it was found that fibers from the PVN mediate at least some of the effects on feeding observed after ventromedial hypothalamus manipulations$^{1,24}$.

PVN SPA could be mediated by either its projections to the pituitary (which releases substances known to produce analgesia upon exogenous administration, e.g. VP, $\beta$-endorphin, and dynorphin), or by its neural projections to other CNS areas, including the spinal cord. The finding that SPA appeared and terminated immediately after stimulation onset and offset, respectively, suggests a neural mediation of this effect. The finding that lower currents were needed to produce SPA from the PVN-Pc than from the PVN-Mg also supports this conclusion, in that PVN-Mg projects mainly to the pituitary, whereas PVN-Pc projects to pain-processing areas in the brainstem and spinal cord$^{15,45,46}$. As many as one third of the total number of hypothalamic neurons projecting directly to the spinal cord originate in the PVN$^{28}$. Many of these fibers terminate in the substantia gelatinosa of the dorsal horn$^{27}$, i.e. near the first central synapse of nociceptive afferents$^{32}$.

The PVN is thought to mediate important components of the stress response, including activation of the hypothalamus–pituitary–adrenal axis$^{12,34}$, release of several stress-related hormones, e.g. vasopressin$^{39}$, prolactin and $\beta$-endorphin$^{39}$, and activation of the autonomic nervous system$^{47}$. Stress is considered a natural trigger for activation of endogenous pain inhibition$^{48}$. Thus, the analgesia produced by PVN stimulation in the present study may reflect the role of this nucleus in mediating the analgesic effects of stress. This hypothesis is supported by the recent finding that PVN lesions reduce cold water swim-induced analgesia$^{49}$.

Studies showing an analgesic response to exogenously administered VP$^{4,6,13,32,33,51}$, an altered pain responsiveness in animals with altered vasopressinergic systems$^{9,10}$, and projections of VP vasopressinergic neurons to brain and spinal cord areas involved in nociception$^{15,45}$ suggest the existence of a VP vasopressinergic pain-modulating system. This system was proposed to serve as a substrate for the non-opioid form of stress-induced analgesia$^{2,10}$. However, the present finding of comparable PVN SPA in LE and DI rats indicates that VP is not essential in mediating the pain-modulating role of the PVN. The elimination of VP-induced analgesia after PVN lesions$^{8}$ and the recent demonstration of vasopressinergic synapses within the PVN$^{14}$ suggest that the PVN itself is the site of VP's analgesic action.

The finding that PVN SPA was not affected by naloxone indicates that opioids are not involved in mediating this analgesia. Neurons within the PVN were shown to synthesize several endogenous opioid peptides, i.e. enkephalins and dynorphin$^{41,52}$ and to regulate $\beta$-endorphin release from the pituitary$^{35}$. Perhaps more selective antagonists would have attenuated PVN SPA. However, the fact that naloxone was ineffective even at the high dose we used (10 mg/kg) makes this possibility unlikely. It was suggested above that the PVN may be involved in mediating the analgesic response to stress. Previous studies demonstrated the existence of both opioid and non-opioid mechanisms of stress-induced analgesia$^{48}$. The PVN may be a substrate for stress-induced analgesia of the non-opioid form.

The results indicate that neuropeptides or neurotransmitters other than vasopressin or opioids mediate PVN SPA. Four other peptides, oxytocin, somatostatin, neuronotensin and CRF are synthesized by PVN neurons that project to the brainstem and spinal cord$^{15,30,45}$. The last 3 of these peptides are known to produce analgesia upon exogenous administration$^{16,17,26,37,41}$. In a preliminary study with a small number of animals, we found that intravenous injections of the oxytocin antagonist...
[d(CH₂)₃Tyr(OMe)², Orn³]-vasotocin², the somatostatin antagonist cyclo(7-aminooctanoyl-Phe-ω-Trp-Lys-Thr-(Bzl)]³ or the neurotensin antagonist [Tyr(Me)²]³-Neurotensin⁴⁶ were ineffective in attenuating PVN SPA even at much higher doses (10–100 times) than required to antagonize some of their effects in the periphery. On the other hand, intravenous administration of the CRF antagonist α-helical CRF⁴⁷ (0.1–0.2 mg/kg) almost doubled SPA threshold for 10–60 min after its administration. These results need to be verified with more subjects, and the role of other PVN neuropeptides should be studied.

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