SEROTONIN INVOLVEMENT IN DESCENDING INHIBITION OF SPINAL NOCICEPTIVE TRANSMISSION PRODUCED BY STIMULATION OF MEDIAL DIENCEPHALON AND BASAL FOREBRAIN¹

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Abstract

The responses of single lumbar dorsal horn neurons to noxious radiant heat stimuli (50°C, 10 sec) applied to glabrous footpad skin were recorded in cats anesthetized with sodium pentobarbital and 70% N₂O. Responses were markedly reduced during electrical stimulation (100-msec trains at 100 Hz, 3/sec, up to 400 μ A) at sites in the medial diencephalic periventricular gray (PVG), preoptic area, and basal forebrain. A role for serotonin (5-hydroxytryptamine, 5-HT) was investigated by determining whether descending inhibition from these areas could be affected by (1) acute systemic administration of the 5-HT antagonist methysergide, or (2) depletion of central 5-HT levels by pretreatment with the 5-HT synthesis inhibitor *p*-chlorophenylalanine (PCPA; 500 mg/kg, i.p.). Inhibition produced by stimulation at these sites was reduced or abolished in 22 cases following administration of methysergide (0.2 to 1 mg/kg) to non-pretreated cats. In the PCPA-pretreated cats, stimulation in preoptic or basal forebrain areas inhibited the responses of 26 units to noxious skin heating to varying degrees; PVG stimulation inhibited the responses of 14 of 26 units, while the remainder were unaffected. The mean current threshold for inhibition produced by PVG or preoptic/basal forebrain stimulation was significantly higher, while mean inhibition at 200 μ A was significantly lower, in units from PCPA-pretreated cats compared to those from non-pretreated cats. The results indicate that 5-HT may be involved in the mediation of spinal inhibition produced by medial diencephalic and basal forebrain stimulation.

Neurons in the spinal dorsal horn are known to be under descending inhibitory influences (Fields and Basbaum, 1978; Willis, 1982). Many dorsal horn neurons in laminae I and IV to VI respond differentially to noxious peripheral stimuli and are thought to participate in the central transmission of nociceptive information (Price and Dubner, 1977). Such neurons are inhibited by electrical stimulation in medial brainstem sites extending from the medullary raphe nuclei through the midbrain periaqueductal gray (PAG) into the medial diencephalic periventricular gray (PVG), preoptic area, and posterior basal forebrain (Oliveras et al., 1974; Fields et al., 1977; Guilbaud et al., 1977; Willis et al., 1977; Belcher et al., 1978; Carstens et al., 1979, 1982; Rivot et al., 1980; Carstens, 1982). Electrical stimulation at similar brain-

stem sites, including the medullary nucleus raphe magnus (NRM; Oliveras et al., 1975), midbrain PAG (Mayer and Leibeskind, 1974; Oliveras et al., 1974), medial diencephalon (Schmidek et al., 1971; Black et al., 1972; Hosobuchi et al., 1977; Rhodes, 1979; Oleson et al., 1980), and medial basal forebrain (Heath and Mickle, 1960; Gol, 1967; Breglio et al., 1970; Mayer and Liebeskind, 1974; Abbot and Melzack, 1978), has been reported to suppress nociceptive responses and human chronic pain. It has been suggested that this so-called stimulation-produced analgesia may be partly mediated via descending inhibition of spinal neurons transmitting nociceptive information (Mayer and Price, 1976).

Considerable evidence supports a role for serotonin (5-hydroxytryptamine, 5-HT) in the mediation of analgesia and descending spinal inhibition produced by medullary NRM and midbrain PAG stimulation (Messing and Lytle, 1977; Willis, 1982). In particular, depletion of central 5-HT levels with the 5-HT synthesis inhibitor *p*-chlorophenylalanine (PCPA), as well as acute blockade of central 5-HT receptors with antagonists such as meth-

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ysergide, reduced or abolished the analgesic (Akil and Liebeskind, 1975; Hayes et al., 1977; Satoh et al., 1980) and inhibitory spinal effect (Rivot et al., 1980; Carstens et al., 1981; Yeziarski et al., 1982) produced by PAG or NRM stimulation. However, little is known concerning the neurotransmitter(s) that mediate(s) descending inhibition and analgesia produced by medial diencephalic and basal forebrain stimulation. Since these latter areas have anatomical connections with midbrain PAG and/or medullary NRM (see "Discussion" for references), it is possible that spinal inhibition produced by diencephalic or forebrain stimulation is mediated via activation of neurons in the PAG and/or NRM, which in turn give rise to inhibitory spinal projections. It would then be expected that the effects of medial diencephalic and basal forebrain stimulation would be mediated at least partly by 5-HT. We have therefore investigated this possibility by determining whether this inhibition is affected by PCPA or the 5-HT antagonist methysergide. An abstract of this work has appeared (Guinan et al., 1982).

Materials and Methods

Electrophysiological experiments were performed using 17 untreated female cats (2.2 to 3.3 kg) and 5 female cats (2.5 to 3.3 kg) pretreated with PCPA (Sigma Chemical Co., suspended in sesame oil; 500 mg/kg, i.p.) 72 hr before the experiment.

The procedures for surgery, stimulation and recording, and histology were identical to those described previously (Carstens, 1982). Briefly, the animals were anesthetized with sodium pentobarbital (Nembutal; 40 mg/kg, i.p.) and artificially ventilated with a gaseous mixture of N₂O and O₂ (12:1) following paralysis. The lumbosacral spinal cord was exposed by laminectomy, and tungsten microelectrodes (12 megohms) were used to record the responses of single dorsal horn units to noxious radiant heating of glabrous footpad skin. Step increases from an adapting temperature of 30°C to the desired end temperature (42 to 52°C) lasting 10 sec were produced at 3-min intervals using a feedback-controlled lamp focused on the skin (Beck et al., 1974). A digital computer was used to construct poststimulus time histograms (PSTHs) of unit responses, which were quantified as the total number of impulses during the 10-sec period starting 5 sec after the onset of heating. Brain stimulation (100-msec trains of 0.1-msec pulses at 100 Hz, 3/sec, amplitude 25 to 400 μ A) was applied via separate concentric bipolar steel electrodes, one of which was stereotaxically lowered into the PVG region (stereotaxic coordinates: anterior, +8 to +12; ventral, +2 to -2, near the midline; Bleier, 1961; Snider and Niemer, 1961) and the other into the medial preoptic area or basal forebrain (anterior, +14 to +16; ventral, +2 to -6, near the midline; Andy and Stephan, 1964). Unit heat-evoked responses without brain stimulation (controls), and again during brain stimulation at each site, were recorded successively. Brain stimulation was begun 10 sec before the onset of heating and lasted 25 sec. The brain stimulation electrodes were left at each position for the duration of the experiment. The effect of PVG, preoptic, or basal forebrain stimulation on a unit heat-evoked response was expressed as a percentage of the control response.

In the PCPA-pretreated cats, the effects of PVG, preoptic, or basal forebrain stimulation at various current intensities on unit 50°C heat-evoked responses, as well as effects of stimulation at one intensity on unit responses to graded noxious heat stimuli, were determined. In the non-pretreated cats the effect of acute systemic administration of methysergide (Sandoz, Inc.; 0.2 to 1 mg/kg, i.v.; duration of injection $\leq$ 2 min) was determined by comparing the magnitude of inhibition produced by brain stimulation of a constant intensity (range: 50 to 300 μ A) before and after the drug injection.

Electrolytic lesions made at the stimulation and recording sites were reconstructed histologically in cresyl violet-stained 40- μ m frozen sections.

Results

Recording and stimulation sites. All units selected gave reproducible high frequency responses to repeated (1/3 min) 50°C heat stimuli. Thirteen of the 17 units studied in the non-pretreated cats receiving acute methysergide were of the class 2 (Handwerker et al., 1975) or wide dynamic range (Price and Dubner, 1977) type, responding to hair movement ($N = 10$) or pressure ($N = 3$) as well as to pinch and noxious heat. Two responded only to noxious pinch and heat (class 3 or nociceptive specific types; Price and Dubner, 1977), and two were not adequately tested with mechanical stimuli. Cutaneous receptive fields spanned one to two toes ($N = 6$) or larger areas ($N = 9$). Units with high mechanical thresholds tended to have smaller receptive fields. Histologically localized recording sites were in laminae IV to VI (Fig. 1A, *open circles*).

In the five cats pretreated with PCPA, 34 units responded to noxious skin heating. However, eight were discarded since they did not respond to subsequent heat stimuli. Similar "habituating" units were reported previously in PCPA-pretreated cats (Carstens et al., 1981). Each of the 26 units giving reproducible responses to heat was of the class 2 or wide dynamic range type, responding at lowest threshold to hair movement ($N = 17$), touch ($N = 2$), or pressure ($N = 5$); two were inadequately tested with mechanical stimuli. There were equal numbers of units with small (one to two toes) and larger receptive fields. Unit recording sites were located in laminae IV to VI (Fig. 1A, *solid circles*).

PVG (Fig. 1B, *circles*) and medial preoptic or basal forebrain (Fig. 1B, *triangles*) stimulation sites are compiled in Figure 1B. Stimulation at each site in non-pretreated cats (Fig. 1B, *open symbols*) markedly reduced the responses of each unit tested. Stimulation at sites in PCPA-pretreated cats (Fig. 1B, *solid symbols*) was less effective, as detailed below.

Methysergide. In the non-pretreated cats, PVG or preoptic/basal forebrain stimulation consistently inhibited responses of dorsal horn units to 50°C heat stimuli as previously reported (Carstens, 1982; Carstens et al., 1982). This is illustrated in Figure 2, showing the response of a dorsal horn unit to the heat stimulus without brain stimulation (*upper left* PSTHs in Fig. 2, A and B) and reduction of the response during medial preoptic or PVG stimulation (*lower left* PSTHs in Fig. 2, A and B, respectively). Following administration of methysergide

Figure 1. Recording and stimulation sites. A, Spinal recording sites for units from PCPA-pretreated cats (●) and from non-pretreated cats (○), compiled on a representative section through the spinal cord at L₆. The lamination scheme of Rexed (1952) is shown to the left. B, Stimulation sites in PCPA-pretreated (solid symbols) and non-pretreated cats (open symbols), compiled on representative frontal sections at the indicated anterior levels. Triangles denote medial preoptic or basal forebrain sites; circles denote PVG sites. The right side is ipsilateral to spinal recording sites. Abbreviations for this and subsequent figures: AC, anterior commissure; cc, corpus callosum; Cd, caudate nucleus; CP, cerebral peduncle; F, fornix; IC, internal capsule; LG, LGN, lateral geniculate nucleus; LV, lateral ventricle; m, mammillary body; ms, medial septal nucleus; MT, mamillothalamic tract; OT, optic tract; PVG, periventricular gray; VB, ventrobasal thalamic nuclei; VL, ventral lateral nucleus.

(0.2 mg/kg, i.v.), the unit's control responses were near the predrug level (*upper middle* PSTHs, Fig. 2, A and B). However, responses were suppressed less during preoptic or PVG stimulation (*lower middle* PSTHs in Fig. 2, A and B). Inhibition recovered within 75 min (*lower right* PSTHs in Fig. 2, A and B). Note that the rebound in unit firing following termination of PVG stimulation increased following methysergide (*lower* PSTHs in Fig. 2B), an effect observed in a few other units. In Figure 3A, inhibition of another unit's response by PVG stimulation was abolished following methysergide, with recovery within 29 min.

Figure 3B summarizes the effect of methysergide to reduce or abolish inhibition produced by PVG (Fig. 3B, circles) or preoptic/basal forebrain stimulation (Fig. 3B, triangles). The degree of inhibition produced by brain stimulation (heat-evoked response during brain stimulation expressed as a percentage of the preceding control) prior to methysergide is plotted against inhibition within 20 min after methysergide for each unit. With both low (Fig. 3B, open symbols) and high doses (Fig. 3B, solid symbols) of methysergide, points lie above the diagonal, indicating that inhibition was reduced or abolished in all cases. The maximal reduction of inhibition was evident within 10 to 30 min following methysergide, and inhibition recovered within 75 min in 10 units but did not recover after more than 60 min in 3 units. In two units from PCPA-pretreated cats, PVG and basal forebrain stimulation inhibited heat-evoked responses, and this inhibition was abolished following methysergide in both cases.

Injection of equal volumes of 0.9% saline had no effect on inhibition. Injection of 0.5 to 1 mg/kg of naloxone in some of these experiments also had no effect on inhibition (unpublished observations). In several cases, inhibition was abolished following methysergide in the absence of blood pressure changes, although reductions in blood pressure (10 to 30 mm Hg) were often observed especially following 1 mg/kg of methysergide.

PCPA. Of the 26 units tested in PCPA-pretreated cats, the heat-evoked responses of each were inhibited by preoptic or basal forebrain stimulation. PVG stimulation did not inhibit the responses of 12 of the 26 units, whereas the remainder were inhibited to varying degrees.

The relationship between brain stimulation intensity and degree of inhibition was investigated in 14 units with PVG stimulation and in 15 units with preoptic or basal forebrain stimulation (Fig. 4). Figure 4A shows the responses of a dorsal horn unit to 50°C heat stimuli without (*upper row* of PSTHs in Fig. 4A) and during PVG stimulation (*lower row* of PSTHs in Fig. 4A) at the indicated current intensities. The same unit's responses (expressed as percentage of control) are plotted against PVG stimulation intensity in Figure 4B, showing that inhibition increased with stimulation intensity, with a threshold for inhibition near 200 μ A. Similar plots are compiled for 14 units with PVG stimulation in Figure 4C and for 15 units with preoptic or basal forebrain stimulation in Figure 4D. Linear regression analysis was performed to calculate the threshold (100% of control) and the slope of the current-inhibition plot (termed the recruitment index; Carstens et al., 1980). Mean thresholds

Figure 2. Reduction by methysergide of descending inhibition produced by medial preoptic or PVG stimulation. *A*, PSTHs (bin width, 0.4 sec) of a dorsal horn unit's responses to 50°C heat stimuli (striped bars) applied to footpad skin, without (CON, upper row) and during 300 μ A stimulation (lower row) at the medial preoptic site shown in the drawing to the right. Non-pretreated cat. Duration of brain stimulation (25 sec) is indicated by the brackets below. Time relative to methysergide administration applies to the lower PSTH in each column. Note that the unit's response was reduced during preoptic stimulation before methysergide (lower left PSTH), but that the response during identical preoptic stimulation was near the control level 18 min after administration of methysergide (0.5 mg/kg, i.v.) (lower middle PSTH). Inhibition partially recovered within 75 min (lower right PSTH). *B*, PSTHs of responses of the same unit in *A* to 50°C heat stimuli, without (CON, upper row) and during PVG stimulation (lower row) at the site shown in the right. Format as in *A*. Note the rebound in unit firing following termination of PVG stimulation (lower middle PSTH) which increased with time (lower right PSTH) after methysergide. Abbreviations are as in Figure 1*B*.

and recruitment indices for PVG or preoptic/basal forebrain stimulation are given in Table I. With both PVG and preoptic/basal forebrain stimulation, mean thresholds were significantly higher, and the recruitment indices were significantly lower, in the PCPA-pretreated cats compared to non-pretreated (control) cats (control data from Carstens, 1982; Carstens et al., 1982). In addition, mean inhibition of unit responses to 50°C heat stimuli produced by PVG or preoptic/basal forebrain stimulation at 200 μ A was significantly less in PCPA-pretreated compared to control groups (Table I).

The effect of PVG or preoptic/basal forebrain stimulation on intensity coding by dorsal horn units of graded noxious heat stimuli was also tested in units from PCPA-pretreated cats (Fig. 5). Figure 5*A* shows a unit's graded responses to heat stimuli at the indicated temperatures, without (CON; left column of PSTHs in Fig. 5*A*) and during PVG stimulation (right column in Fig. 5*A*). This unit's integrated responses with (Fig. 5*B*, open circles) and without (control; Fig. 5*B*, solid circles) PVG stimulation are plotted against skin stimulus temperature in

Figure 5*B*. During PVG stimulation, the slope of the linear temperature-response function was reduced (to 63% of control) with a 2.3°C increase in the extrapolated firing threshold. Figure 5*C* similarly plots the mean responses of 11 units without (control; solid circles, Fig. 5*C*) and with (open circles, Fig. 5*C*) PVG or preoptic/basal forebrain stimulation (100 to 300 μ A). The mean slope reduction produced by brain stimulation was to 67% of control, and the mean threshold was increased by 1.9°C.

Discussion

Inhibition of spinal dorsal horn neuronal responses to noxious skin heating produced by stimulation of PVG, medial preoptic area, and posterior basal forebrain was reduced or abolished following systemic administration of the 5-HT antagonist, methysergide, and was significantly weaker in animals whose central 5-HT levels had been depleted by pretreatment with PCPA. These results indicate that 5-HT may play a role in mediating descend-

Figure 3. Reduction or blockade of descending inhibition by methysergide. **A**, PSTHs of another unit's responses to 50°C heat stimuli, without (*Control*, upper row) and during (*lower row*) PVG stimulation at the site shown to the right. Format as in Figure 2. **B**, The graph plots 50°C heat-evoked response of each dorsal horn unit during brain stimulation (expressed as percentage of control response in the absence of brain stimulation) before methysergide (*abscissa*) against response of the same unit during brain stimulation within 20 min after administration of methysergide (*ordinate*). Each point represents a separate unit. The dashed diagonal line indicates equal inhibition before and after methysergide. $\blacktriangle$, $\triangle$: medial preoptic or basal forebrain stimulation; $\bullet$, $\circ$: PVG stimulation. Open symbols, low dose of methysergide (0.2 to 0.5 mg/kg); solid symbols, 1 mg/kg dose of methysergide. Abbreviations are as in Figure 1B.

ing inhibition from these areas, within the limitations of the specificity of the drug treatments as discussed below.

Methysergide. There is considerable evidence that the peripheral 5-HT antagonist methysergide also acts centrally. Systemic administration of methysergide and other putative 5-HT antagonists (metergoline, cinanserin, cyproheptadine) blocked inhibition produced by midbrain PAG stimulation of spinal neuronal responses to noxious skin input (Carstens et al., 1981; Yeziarski et al., 1982), blocked bulbospinal inhibition of monosynaptic reflexes (Clineschmidt and Anderson, 1970; but see Sinclair and Sastry, 1974), blocked dorsal root potentials evoked by medullary NRM stimulation (Proudfit et al., 1980), and blocked analgesia produced by stimulation in or opiate microinjection into the midbrain PAG or medullary NRM (Yaksh et al., 1976; Hayes et al., 1977; Satoh et al., 1980).

Methysergide presently reduced or abolished descending inhibition produced by medial diencephalic and basal forebrain stimulation. This cannot be ascribed to a general systemic effect such as hypotension, since methysergide frequently blocked inhibition in the absence of blood pressure changes. That methysergide did not uniformly abolish inhibition may have been due to coacti-

vation of a nonserotonergic inhibitory system (see below). Blockade of inhibition occurred within 20 min, and inhibition recovered within 75 min in many, but not all, units. Inhibition of spinothalamic neurons produced by PAG stimulation only partially recovered following its blockade by larger doses of methysergide (2 to 5 mg/kg) (Yeziarski et al., 1982).

The site of action of methysergide cannot be determined from experiments using the systemic route of administration. One possible site is in the spinal dorsal horn, in which are located 5-HT-containing synaptic terminals originating from neurons in NRM (Ruda and Gobel, 1980). 5-HT has a depressant action on dorsal horn neurons (Randić and Yu, 1976; Belcher et al., 1978; Headley et al., 1978). Methysergide antagonized the depressant action on dorsal horn neurons of 5-HT, but not noradrenaline, administered into the substantia gelatinosa (Griersmith and Duggan, 1980), suggesting possible specificity of methysergide for 5-HT receptors. 5-HT antagonists most effectively blocked bulbospinal reflex inhibition when injected into the arterial supply of the spinal cord (Clineschmidt and Anderson, 1970). On the other hand, methysergide failed to reduce inhibition of spinal dorsal horn neurons produced by NRM stimula-

Figure 4. Recruitment of descending inhibition by graded increases in brain stimulation intensity in PCPA-pretreated cats. **A**, PSTHs (bin width, 0.4 msec) of dorsal horn unit's responses to repeated 50°C heat stimuli (bars below), without (CONT., upper row) and during concomitant PVG stimulation (lower row) at the indicated current intensities. From a PCPA-pretreated cat. Brain stimulation began 10 sec before onset of the heat stimulus and lasted 25 sec. **B**, The graph plots magnitude of 50°C heat-evoked response (expressed as percentage of control) against intensity of PVG stimulation for the same unit shown in **A**. PVG stimulation site is shown in the inset (for abbreviations, see Fig. 1B). **C**, Graph as in **B**, plotting the responses of 14 units from PCPA-pretreated cats against PVG stimulation intensity. **D**, Graph as in **C**, plotting the responses of 15 units from PCPA-pretreated cats against intensity of medial preoptic or basal forebrain stimulation.

tion in the cat (Belcher et al., 1978; Griersmith et al., 1981) and only weakly reduced inhibition of primate spinothalamic neurons produced by NRM stimulation, in contrast to methysergide's strong blockade of inhibition produced by PAG stimulation (Yeziarski et al., 1982). Instead of a spinal site of action, methysergide could disrupt a potential excitatory serotonergic projection to NRM from the dorsal raphe nucleus (Yeziarski et al., 1981), although such a projection has not been found in the cat (Abols and Basbaum, 1981). It was recently reported that 5-HT inhibits, rather than excites, raphe-spinal neurons in the monkey (Willcockson et al., 1982). The site of action of methysergide thus remains uncertain.

PCPA. PCPA selectively inhibits 5-HT synthesis (Koe and Weissman, 1966) and, at doses comparable to those used presently (500 mg/kg or two doses of 300 mg/kg), reduced 5-HT levels to 40% of control in the brainstem (Keller, 1972) and to 16 to 54% of control in the cat's thoracic spinal cord (Coote et al., 1978). We found descending inhibition from the medial diencephalon and basal forebrain to be weaker, but not absent, in PCPA-pretreated cats (Table I). Inhibition in PCPA-pretreated cats may have been mediated by residual 5-HT and/or by coactivation of a nonserotonergic descending inhibitory system. That inhibition in PCPA-pretreated cats was blocked following administration of methysergide in two units might be interpreted as evidence favoring the former possibility. Alternatively, it was previously reported that stimulation in the lateral midbrain reticular formation (LRF) inhibited dorsal horn unit responses to noxious skin heating (Carstens et al., 1980) and that this inhibition was not reduced by PCPA (Carstens et al., 1981). LRF stimulation resulted in parallel rightward shifts in unit intensity coding functions for graded noxious heat stimuli with increases in the neuronal firing threshold, similar to the present effect of brain stimulation in PCPA-pretreated cats (Fig. 5). It is therefore possible that the higher stimulation intensities needed to produce inhibition in the PCPA-pretreated cats may have coactivated the nonserotonergic inhibitory LRF system. In this context, neurons in the PVG and medial preoptic area have been reported to project to the mid-

TABLE I

Comparison of quantitative parameters for inhibition produced by PVG and medial preoptic or basal forebrain stimulation in untreated control cats^a and cats pretreated with PCPA

	Control			PCPA (500 mg/kg)		
	Mean	SD	N	Mean	SD	N
PVG Stimulation						
Mean inhibition (% control) at 200 μ A	41.7	31.3	15	67.2 ^b	37.1	14
Mean threshold (μ A) for inhibition	67.4	67	14	144.9 ^b	127.5	13
Recruitment index (% inhibition/100 μ A)	46	25	15	23.5 ^b	30	14
Mean current (μ A) at 50% inhibition	170.1	79.2	14	266.2 ^{b,c}	130.8	13
Medial Preoptic/Basal Forebrain Stimulation						
Mean inhibition (% control) at 200 μ A	31	21.7	14	62.9 ^b	19.2	15
Mean threshold (μ A) for inhibition	25.3	18.7	15	98.4 ^b	26.5	15
Recruitment index (% inhibition/100 μ A)	41	28	14	25 ^b	15	15
Mean current (μ A) at 50% inhibition	126.1	52.9	13	233.9 ^{b,c}	70.3	15

^a Data from Carstens, 1982, and Carstens et al., 1982.

^b Significantly different from control ($p < 0.05$, t test).

^c Values represent underestimates, since four units in each group were not inhibited at currents above the maximum used (400 μ A) for this calculation.

Figure 5. Effect of PVG stimulation on intensity coding for graded noxious heat stimuli by dorsal horn units in PCPA-pretreated cats. **A**, PSTHs (bin width, 0.4 sec) of dorsal horn unit's response to heat stimuli at the indicated temperatures, without (CON, left column of PSTHs) and during concomitant PVG stimulation (right column). At each randomly selected temperature, controls alternated with brain stimulation trials at 3-min intervals. From a PCPA-pretreated cat. **B**, The graph plots integrated responses of the same unit as in **A** against temperature of heat stimulus, without (●, control) and during PVG stimulation (○). Lines were calculated by linear regression analysis. PVG stimulation sites are shown in the inset (for abbreviations, see legend to Fig. 1B). **C**, Graph as in **B**, plotting mean responses of 11 units against temperature of heat stimulus without (●, control) and during PVG, medial preoptic, or basal forebrain stimulation (○).

brain reticular formation in the cat (Parent and Steriade, 1981). It is also possible that neurons mediating descending inhibition release one or more inhibitory transmitter(s) in addition to 5-HT, such that depletion of 5-HT would weaken but not abolish inhibition.

Pathways for inhibition. Descending inhibition from the PVG may have been mediated via direct spinal projections originating in posterior (Kuypers and Maisky, 1975; Basbaum and Fields, 1979) or paraventricular (Hancock, 1976; Hosoya, 1980) hypothalamic nuclei, or indirectly via connections with the midbrain PAG (Conrad and Pfaff, 1976b; Grofova et al., 1978; Saper et al., 1979) and/or medullary NRM (Abols and Basbaum, 1981). Our results indicate that 5-HT may be involved in this inhibition, yet 5-HT-containing neuronal cell bodies were not found in the diencephalon, forebrain, or spinal cord in a recent extensive study (Steinbusch, 1981). The implication is that the PVG must be functionally linked with 5-HT-containing neurons located in the dorsal raphe nucleus in and ventral to the PAG, and/

or in the pontomedullary raphe nuclei including NRM (Steinbusch, 1981). Several observations suggest that the PAG may mediate descending inhibition from the PVG: (1) PVG and PAG stimulation produces parametrically similar inhibition of spinal neurons (Carstens et al., 1979; Carstens, 1982), and inhibition is blocked by methysergide and PCPA; (2) stimulation in the medial diencephalon predominantly excites PAG neurons (Sakuma and Pfaff, 1980); and (3) analgesia produced by medial diencephalic stimulation is blocked by PAG lesions, whereas analgesia produced by PAG stimulation is not blocked by medial diencephalic lesions (Rhodes, 1979). Descending inhibition from the PAG may, in turn, be mediated via activation of neurons in NRM (Fields and Anderson, 1978; Lovick et al., 1978) which project in the dorsolateral funiculi to terminate in the spinal dorsal horn (Basbaum et al., 1978). Double-label studies have shown that many raphe-spinal projection neurons also contain 5-HT (Bowker et al., 1981). The inhibitory spinal effect of NRM stimulation is abolished in PCPA-pretreated animals (Rivot et al., 1980), and raphe-spinal inhibition and analgesia resulting from PAG stimulation are blocked by dorsolateral funicular lesions (Basbaum et al., 1977; Fields et al., 1977; Willis et al., 1977). In summary, descending inhibition from the PVG might involve a serial PAG-NRM-spinal pathway.

5-HT-containing neurons were recently reported in the PVG region of rats pretreated with pargyline and L-tryptophan (Frankfurt et al., 1981). Such neurons could conceivably mediate descending inhibition, but it is not known whether they give rise to descending projections.

Descending inhibition produced by stimulation of the medial basal forebrain could be mediated via a direct projection to the dorsal raphe/PAG (Aghajanian and Wang, 1977) or via connections with the medial preoptic area (Swanson and Cowan, 1979; Krayniak et al., 1980), which in turn connects with more posterior areas of the medial hypothalamus and midbrain (Conrad and Pfaff, 1976a; Sakai et al., 1977; Beitz, 1982). It was recently reported that medial septal stimulation excited some neurons in the PAG (DeNasi and Thomas, 1982). Alternatively, inhibition from the medial basal forebrain may involve a projection to the lateral hypothalamic area (Swanson and Cowan, 1979; Krayniak et al., 1980); stimulation of which also inhibits dorsal horn neurons (Carstens et al., 1983). The PAG and NRM receive projections from the lateral hypothalamic area (Abols and Basbaum, 1981).

One final possibility is that inhibition could be mediated via antidromic activation of neurons in NRM or the dorsal raphe nucleus from more anterior stimulation sites. Raphe neurons give rise to extensive ascending projections (Moore et al., 1978; Kohler et al., 1982); conceivably, some may give off both ascending and descending collaterals.

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