Time Course and Effective Sites for Inhibition from Midbrain Periaqueductal Gray of Spinal Dorsal Horn Neuronal Responses to Cutaneous Stimuli in the Cat*

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Summary. Inhibition of spinal dorsal horn neuronal responses to noxious (50°C) skin heating by stimulation of the midbrain periaqueductal gray (PAG) was quantitatively investigated in cats anesthetized with sodium pentobarbital and nitrous oxide. Systematic variation of the interval between onset of PAG stimulation (PAGS) and onset of noxious skin heating revealed that a marked reduction of spinal unit heat-evoked discharges occurred immediately upon onset of PAGS, and ceased immediately at offset of PAGS with a post-stimulation excitatory rebound. Stimulation at sites in both ventral and dorsal PAG produced inhibition, the strength of which increased sometimes in a linear manner with increasing strength of PAGS. Thresholds for the generation of descending inhibition were higher in dorsal than ventral PAG. PAGS also inhibited spinal unit responses to non-noxious skin stimulation (brushing of hairs). Descending inhibition from PAG is considered as a possible mechanism for analgesia produced by stimulation of PAG and other brainstem structures.

Key words: Periaqueductal gray – Spinal dorsal horn – Descending inhibition – Noxious heat – Analgesia

Electrical stimulation of the midbrain periaqueductal gray (PAG) inhibits the responses of spinal dorsal horn neurons to noxious skin stimulation (Oliveras et al., 1974). We have recently shown that noxious radiant skin heating evokes discharges in spinal dorsal horn neurons which are reproducible during a repetitive series of heat stimuli, and that these heat-evoked discharges are strongly inhibited by stimulation of PAG (Carstens et al., 1979d). In the present study, we have quantitatively investigated the time course for this inhibition, as well as the effective sites throughout the PAG for evoking inhibition. Selectivity of inhibition by PAG stimulation for unit responses to noxious versus non-noxious stimuli was also investigated.

Methods

Cats weighing 2.1–3.2 kg were anesthetized with sodium pentobarbital (Nembutal, 40 mg/kg i.p.). The lumbar-sacral spinal cord and left superficial peroneal (SP) and posterior tibial (PT) nerves were surgically exposed. Arterial blood pressure, end-tidal CO2, and rectal temperature were monitored and maintained within normal limits. Animals were paralyzed (Pancuronium bromide, 0.4 mg/kg i.v.) and artificially ventilated with a gaseous mixture of N2O and O2 (3:1) to maintain anesthesia. Halothane was added if pupillary dilatation and blood pressure increases were evoked by noxious stimulation (Carstens et al., 1979c, d).

Glass micropipettes filled with 3 M KCl were used to record single units in the lumbar cord region receiving maximal input from the SP and PT nerves (Gregor and Zimmermann, 1972). Only units responding to electrical stimulation of SP and/or PT at both A-fiber (2 V, 0.1 ms pulse duration) and C-fiber (30 V, 1 ms pulse duration) strengths (Gregor and Zimmermann, 1972) were selected for study. Noxious radiant heat stimuli were applied to the skin of the ipsilateral hindfoot with a feedback-controlled lamp (details in Beck et al., 1974). Non-noxious mechanical stimuli were applied by moving hairs of the foot with a motor-controlled hydraulic piston to which a brush was attached. Midbrain stimulation was applied through a concentric bipolar steel electrode which was stereotaxically positioned in the PAG (AP 0, DV 0) and left in place for the duration of the experiment. PAG stimulation consisted of constant current pulse trains (pulse duration 0.1 ms, frequency 100 Hz, train duration 100 ms, repetition rate 300 ms, intensity 50–900 μA). Estimates of current spread using these parameters have been provided elsewhere (Carstens et al., 1979d).

At the conclusion of an experiment, an electrolytic lesion was made at the PAG stimulation site. The recording microelectrode was cut off and left in situ in the last track. The cat was perfused with 1% potassium ferrocyanide followed by 10% formalin. Stimulation sites in midbrain and microelectrode tracks in the cord were localized in 40 μm sections stained with cresyl echt violet. The depth of the microelectrode tip from the cord dorsum was coordinated with the histologically localized microelectrode track to determine the spinal unit recording site. Further methodological details were given elsewhere (Carstens et al., 1979c).
Results

Unit Locations and Receptive Field Properties

A total of 43 units was studied in 27 cats. All were inhibited by PAG stimulation (PAGS). The locations of 40 units have been compiled in Fig. 1 on a representative section of the spinal cord at L7. Almost all units were located in the area corresponding to Rexed (1952) laminae IV and V, with a few ventral to lamina V. Two were located in the marginal zone of the dorsal horn. Recording sites were restricted to the dorsal horn since the microelectrode penetrations were made no deeper than 2.5 mm from the cord dorsum.

Thirty-six units were characterized according to adequate natural skin stimuli and size of receptive field. All units responded to strong cutaneous stimuli, such as pressure, pinch, and/or noxious heating. Of these, 42% received additional input from hair follicle receptors, and these units were characterized by light mechanical stimuli (touch, tap). Such units have been called “wide dynamic range” (Price and Dubner, 1977) or “class 2” (Iggo, 1974; Handwerker et al., 1975) units. Thirty-three percent of the units had only high threshold mechanical input (class 3, according to Iggo, 1974). One of these latter units was located in the marginal zone (the other marginal zone unit was not characterized according to adequate stimuli).

Cutaneous receptive fields varied in size. Small receptive fields spanning one or two toes were seen in 40% of the units, while the remainder had larger receptive fields spanning 3 or more toes and often encompassing the entire paw. Units having large and small receptive fields were approximately equally distributed among the adequate stimulus categories.

Time Course of PAGS Inhibition

The time course of inhibition of 50°C heat-evoked unit responses by PAGS was quantitatively determined in six experiments. The method is illustrated for a typical unit in Fig. 2A. PAGS was always applied for a duration of 40 s as indicated by the bar above the graph. The 50°C heat stimulus lasted 10 s and was applied at variable intervals following the onset of PAGS. Unit responses were expressed as the total number of impulses in the 10 s spike analysis period (upper bar in Fig. 2A) starting 5 s after the onset of the heat stimulus. The graph in Fig. 2A plots the unit’s responses to 50°C heating at various intervals after the onset of 450 µA PAGS. Strong inhibition was observed when the heat stimulus was applied concurrently with the onset of PAGS. A similar level of inhibition was observed when the heat stimuli were applied at greater intervals after the onset of PAGS. When the heat stimulus was applied 5 s before the offset of PAGS (spike analysis period thus starting concurrently with PAGS offset), no inhibition was observed. A rebound effect occurred shortly after offset of PAGS, and responses were again at the pre-PAGS level 3 min later. The mean time course of PAGS inhibition of 6 units is shown in Fig. 2B. A post-PAGS rebound was seen in all units tested, increasing in degree with greater PAGS current strength. Long-term inhibitory effects of PAGS were not observed for any of the units, but have been observed in 3 units reported previously (Carstens et al., 1979d).

Recruitment of Inhibition by Stimulation of Ventral and Dorsal PAG

Recruitment of inhibition by varying PAGS current strengths was investigated for 32 units in 27 experiments. The PAG stimulation sites were histologically localized either in the ventral (N = 13 experiments) or dorsal (N = 14) PAG, and have been compiled on a representative section through the midbrain at AP 0 in Fig. 3 (inset). Recruitment curves, which plot the degree of inhibition of 50°C heat-evoked spinal unit responses versus PAGS current strength, are shown for all units in Fig. 3. The left-hand graph plots recruitment curves for 14 units inhibited by ventral PAGS, and the right-hand graph, those for 18 units inhibited by dorsal PAGS. Comparing the degree of inhibition by ventral versus dorsal PAGS, it appears that the recruitment curves for dorsal PAGS are scattered over a larger range of current strengths. This is quantitatively supported by comparison of (1) the mean threshold for inhibition, which was signifi-