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Short communication

Blockade of $GABA_A$ receptors in the midbrain periaqueductal gray abolishes nociceptive spinal dorsal horn neuronal activity

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Single spinal dorsal horn neuronal responses to noxious skin heating or innocuous skin brushing were recorded in pentobarbital aneasthetized rats. The heat-evoked activity was selectively abolished by blockade of $GABA_A$ receptors in the midbrain periaqueductal grey (PAG) by microinjections of 40 or 400 pmol bicuculline. It is concluded that antinociceptive output neurons in the PAG that trigger descending inhibition are maximally active when released from tonic GABAergic inhibition.

Pain; Analgesia; Bicuculline; (Descending inhibition, Rat)

1. Introduction

Electrical stimulation or morphine microinjections into the midbrain periaqueductal grey (PAG) leads to analgesia. This is partly attributable to activation of inhibitory systems which descend through various aspects of the spinal cord white matter (Sandkühler et al., 1987b) to all levels of the spinal cord (recently reviewed by Besson and Chaouch, 1987; Gebhart, 1988). The activation of antinociceptive output neurons in the PAG by predominantly inhibitory substances such as morphine could be explained by their disinhibition (Moreau and Fields, 1986; Depaulis et al., 1987) e.g. by release from tonic GABAergic inhibition. The role of GABA in antinociceptive mechanisms has been reviewed recently (Sawynok, 1987).

We report here that blocking $GABA_A$ receptors in restricted regions of the PAG by pmol doses of bicuculline maximally activates descending antinociceptive systems.

2. Materials and methods

Male Sprague-Dawley rats weighing 200-300 g were used and were deeply aneasthetized with sodium pentobarbital (50 mg/kg i.p. initially and 6 mg/kg per h i.v for maintenance). Extracellular recordings were made from single neurons in the dorsal horn of the lumbar spinal cord. All neurons responded to noxious radiant skin heating (50 °C, 10 s) and to innoucuous brushing of the glabrous skin of the ipsilateral hindpaw.

A multibarrel glass pipette (tip diametre $\leqslant 40$ μm) was filled with 0.4 and 4.0 mM bicuculline methiodide (Sigma), pH adjusted to 2.5 with HCl, and fast green dye (Chroma). One pipette contained 3-7 carbon fibers (Sigri, 7 μm diametre) for monopolar electrical stimulation (Willmann and Sandkühler, 1988). In some experiments one pipette was filled with saline (NaCl, 0.9%, pH adjusted to 2.5). The array was lowered into the

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midbrain (P 7.5-5.0, L 0.0-3.0, H 4.0-8.0) with a stepping motor in steps of 250 or 500 μm.

Electrical stimulation (125-500 μA, 0.1 ms cathodal pulses given at 100 Hz) was used to identify the effective sites from which descending inhibitory systems were activated. Bicuculline was microinjected at these sites in volumes of 100 nl. Injection sites were later labelled with fast green dye and were located histologically on 20 μm thick transverse sections through the midbrain. Evoked responses are expressed as the total number of impulses in 15 s beginning from the start of the stimulus and are corrected for spontaneous activity. Mean values are given with S.D.s.

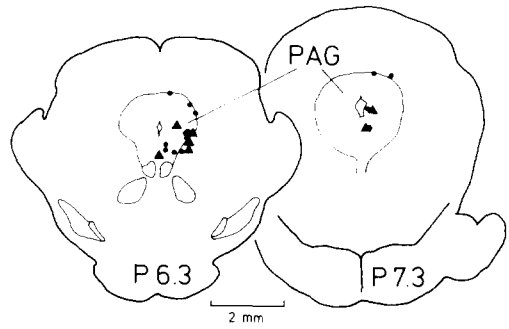

Fig. 2. Sites in the periaqueductal grey (PAG) at which blockade of $GABA_A$ receptors abolished the noxious heat-evoked responses of spinal dorsal horn neurons. The 21 injection sites from 14 animals are overlayed on representative coronal sections through the midbrain 6.3 and 7.3 mm posterior to bregma, respectively. Bicuculline methiodide was injected in quantities of 40 (●) or 400 pmol (▲).

3. Results

Twenty-one injection sites were identified in the midbrains of 14 rats at which bicuculline (40 or 400 pmol) abolished the noxious heat-evoked responses of 19 spinal dorsal horn neurons, see fig. 1A for a typical example. All sites were located within or close to the boundaries of the PAG, either at the dorsal edge or in the ventral quadrant of the PAG (fig. 2). Less effective sites (mean reduction to 50.3 ± 23.3% of control, mean ± S.D., n = 41) were found throughout the PAG and adjoining ventral tegmentum. All 15 injection sites tested in the lateral reticular formation neighbouring the PAG and 12 sites within the PAG were ineffective. Inhibition by 40 pmol bicuculline was fully reversible within 2-6 min after the injection, while 400 pmol abolished the heat-evoked responses for up to 30 min, with a gradual recovery to control values within 40 min post-injection. Inhibition was often accompanied by tachypnea and movements of the whiskers. The responses

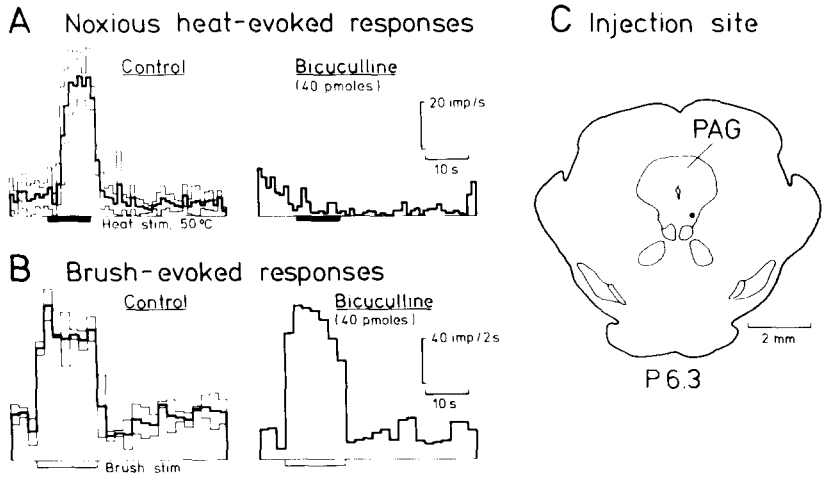

Fig. 1. The descending inhibition elicited by microinjection of bicuculline into the PAG is selective for the noxious, heat-evoked responses of spinal dorsal horn neurons (A) but not for the innocuous, brush-evoked responses (B). Peristimulus-time-histograms (bin width in A: 1000 ms, in B: 2000 ms) of one typical example are averaged for 3 control responses (±S.D.) to enable comparison with individual responses 1 min after the injection of 40 pmol bicuculline methiodide at the site shown in (C).

evoked by gentle skin brushing, however, were largely unaffected by microinjection of bicuculline at the same sites, see fig. 1B for a representative example. In 10 experiments in 7 rats the microinjection of saline (100 nl, pH 2.5) failed to affect the heat-evoked responses (102 ± 33% of control), while electrical stimulation (125 μA) at the same sites significantly reduced the responses fo 45.4 ± 26.6% of control.

4. Discussion

To our knowledge this is the first report showing that supraspinal chemical stimulation in pmol doses may maximally activate the descending inhibition of spinal nociceptive neurons. The blockade of $GABA_A$ receptors within well defined regions of the PAG selectively abolished the noxious heat-evoked responses in the spinal dorsal horn. This finding is consistent with a report by Moreau and Fields (1986), who showed that bicuculline injected into the ventral quadrant of the PAG inhibits the spinally mediated, nocifensive tail-flick reflex in rats. Further, Depaulis et al. (1987) reported that the descending inhibition of the tail-flick reflex induced by microinjection of morphine into the PAG is reversed by subsequent injections of the GABA agonist 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin 3-ol (THIP) and is potentiated by injections of the GABA antagonist picrotoxin. Taken together, these studies provide convergent evidence that antinociceptive output neurons in the PAG are subject to a powerful tonic inhibition mediated by $GABA_A$ receptors. The antinociception by morphine (and other predominantly inhibitory substances) in the PAG may thus be due to the inhibition of GABAergic interneurons in the PAG, thereby disinhibiting the antinociceptive output neurons. This hypothesis is consistent with findings that the descending inhibition elicited by morphine microinjections (Ossipov and Gebhart, 1984), but not by focal electrical stimulation (Sandkühler et al., 1987a), into the PAG is attenuated by the barbiturate pentobarbital. Since pentobarbital may enhance GABAergic inhibition (e.g. Olsen, 1982), it may mask the disinhibition of output neurons produced by morphine microinjections. Pentobarbital would be expected to have no effect if the output neurons are directly activated by electrical current.

The responses to heating the hindpaw were virtually abolished, even at the lower of the two doses of bicuculline tested. Supraspinal morphine has not been reported to completely inhibit the heat-evoked responses of cat (Gebhart et al., 1984) or rat (Gebhart and Jones, 1988) spinal dorsal horn neurons. This apparent greater efficacy of bicuculline may indicate that (i) GABAergic interneurons in the PAG are not completely inhibited by microinjected morphine, (ii) some GABAergic inhibition may arise from neurons located outside the PAG, e.g. in the medial hypothalamus (Sander et al., 1981) or the reticular nucleus of the thalamus (Wiberg and Rinvik, 1988), (iii) that morphine may also directly inhibit antinociceptive output neurons in the PAG, thereby masking the effect of disinhibition.

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References

Besson, J.-M. and A. Chaouch, 1987, Peripheral and spinal mechanisms of nociception, Physiol. Rev. 67, 67.

Depaulis, A., M.M. Morgan and J.C. Liebeskind, 1987, GABAergic modulation of the analgesic effects of morphine microinjected in the ventral periaqueductal gray matter of the rat, Brain Res. 436, 223.

Gebhart, G.F., 1988, Descending inhibition of nociceptive transmission, in: Basic Mechanisms of Headache, eds. Olesen, J. and L. Edvinsson (Elsevier, Amsterdam) p. 201.

Gebhart, G.F. and S.L. Jones, 1988, Effects of morphine given in the brainstem on the activity of dorsal horn nociceptive neurons, in: Descending Brainstem Controls of Nociceptive Transmission, Prog. in Brain Res., Vol. 77, H.L. Fields and J.-M. Besson (Elsevier, Amsterdam) p. 231.

Gebhart, G.F., J. Sandkühler, J.G. Thalhammer and M. Zimmermann, 1984, Inhibition in spinal cord of nociceptive information by electrical stimulation and morphine microinjection at identical sites in midbrain of the cat, J. Neurophysiol. 51, 75.

Moreau, J.-L. and H.L. Fields, 1986, Evidence for GABA involvement in midbrain control of medullary neurons that modulate nociceptive transmission, Brain Res. 397, 37.

Olsen, R.W., 1982, Drug interactions at the GABA receptor-ionophore complex, Ann. Rev. Pharmacol. Toxicol. 22, 245.

Ossipov, M.H. and G.F. Gebhart, 1984, Light pentobarbital anesthesia diminishes the antinociceptive potency of morphine administered intracranially but not intrathecally in the rat, European J. Pharmacol. 97, 137.

Sander, G., D. Dessort, P. Schmitt and P. Karli, 1981, Distribution of GABA in the periaqueductal gray matter. Effects of medial hypothalamic lesions, Brain Res. 224, 279.

Sandkühler, J., Q.-G. Fu, C. Helmchen and M. Zimmermann, 1987a, Pentobarbital, in subanesthetic doses, depresses spinal transmission of nociceptive information but does not affect stimulation-produced descending inhibition in the cat, Paion 31, 381.

Sandkühler, J., Q.-G. Fu and M. Zimmermann, 1987b, Spinal pathways mediating tonic or stimulation-produced descending inhibition from the periaqueductal gray or nucleus raphe magnus are separate in the cat, J. Neurophysiol. 58, 327.

Sawynok, J., 1987, GABAergic mechanisms of analgesia: an update. Pharmacol. Biochem. Behav. 26, 463.

Wiberg, M. and E. Rinvik, 1988, A reciprocal connection between the periaqueductal grey matter and the reticular nucleus of the thalamus an anatomical study in the cat, European J. Neurosci. 1 (Suppl. 1), 175.

Willman,, E. and J. Sandkühler, 1988, A 40 μm tip device for electrical and pharmacological stimulation, extracellular recording and reversible blockage at identical sites within the CNS. European J. Neurosci. 1 (Suppl. 1), 182.