Microdialysis of excitatory amino acids in the periaqueductal gray of the rat after unilateral peripheral inflammation

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Summary. This study measured the release of glutamate (Glu) and aspartate (Asp) amino acid transmitters in the ventrocaudal compartment of the rat periaqueductal gray (PAG) following exposure to unilateral peripheral inflammation. The release of endogenous Glu and Asp from the rat ventrocaudal PAG was monitored with the microdialysis technique in unanesthetized, unrestrained rats. There was significant increase (1,300%) in the basal concentrations of Glu release in the 7 days Complete Freund's Adjuvant (CFA) treated group compared to 24h mineral oil control group. Amino acid release was induced by infusing veratridine (75 μM, a sodium channel activator) directly through the 1 mm long dialysis probe. Perfusion of veratridine into the ventrocaudal PAG resulted in significant elevation of Glu and Asp amino acids. In the 24h and 7 days CFA treated rats, veratridine-evoked release of Glu was significantly decreased in the lateral ventrocaudal PAG compared to control rats injected with mineral oil (CFA vehicle). The peak minus baseline concentrations of Glu in 24h and 7 days CFA treated groups decreased 55.7% and 43.9%, respectively. In contrast, the basal and the peak minus baseline concentrations of Asp showed no significant change between control group and 24h and 7 days CFA treated animals. The results provide direct evidence that Glu excitatory amino acid may be involved in nociception/nociception modulation pathway in the ventrocaudal PAG.

Keywords: Amino acids – Analgesia – Aspartate – Glutamate – Nociception – Pain

Introduction

The midbrain periaqueductal gray (PAG) is one of the major sites participating in pain modulation process via descending multisynaptic pathways that project to spinal cord (Basbaum and Field, 1984). The most sensitive portion
of this midbrain region to antinociception is the ventrocaudal PAG (Fang et al., 1989). Electrical stimulation or morphine microinjection into this portion of the PAG have been shown to produce analgesia accompanied by a strong inhibition of dorsal horn neurons which respond to noxious stimuli (Reynolds, 1969; Bennet and Mayer, 1979). The role played by the PAG in the above function is dependent on the interconnections of this midbrain area with the other regions of the central nervous system, particularly, the direct projection from the PAG to the spinal cord (Holstege and Kuypers, 1982), and the PAG-nucleus raphe magnus (NRM)-spinal cord projection pathway which is the major descending modulation pathway of nociception (Fields and Anderson, 1978). Inputs from laminae I and II and the lateral cervical nucleus of the spinal cord project directly to the lateral portion of the caudal PAG (Keay and Bandler, 1993).

Excitatory amino acid (EAA) glutamate (Glu) appears to play a significant role in the midbrain PAG. Microinjections of Glu into the rat PAG produced a potent analgesia and increased the threshold of a flexion reflex elicited by thermal stimuli to the hindpaw. It also caused excitation of neurons in the NRM (BehBehani and Fields, 1979). Furthermore, Jacquet (1988) has shown that EAA injection into the PAG produced a potent analgesia that is antagonized by prior injection of the N-methyl-D-aspartate (NMDA) antagonist suggesting that Glu may produce analgesia by activating directly the PAG projection neurons which influence the descending antinociception pathways. This analgesia is thought to be produced as a result of Glu acting on excitatory amino acid receptors.

Although many studies (Aimone and Gebhart, 1986; Wiklund et al., 1988; Beitz, 1990; Beitz and Williams, 1991) show that PAG neurons and axon terminals contain Glu and Asp, and suggest that EAA may be important neurotransmitters in the PAG-NMR pain modulation projection pathway, it is not clear whether or not these two amino acids exhibit differential release in the PAG in response to nociceptive stimuli. In the present study, an in vivo microdialysis procedure was utilized, which represents an important tool for monitoring extracellular levels of amino acids within specific regions of the brain in awake, freely moving animals (Renno et al., 1992). It allows analysis of extracellular levels of amino acids before, during and after stimulation of the PAG without the complicating effect of anesthetics (Benveniste and Huttemeier, 1990; DiChiara, 1990). Thus, using the microdialysis technique, this study was designed to determine the Glu and Asp basal concentrations and veratridine induced release concentrations in the ventrocaudal PAG in response to unilateral peripheral inflammation as a model for acute nociception in rats.

**Materials and methods**

*Animals and treatment regimen*

All animals used in this study were adult (225-350 g), male Sprague-Dawley rats. All surgical procedures were performed under ketamine/xylazine anesthesia (1 ml ketamine: