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Research report

Patterns of fos expression in the rostral medulla and caudal pons evoked by noxious craniovascular stimulation and periaqueductal gray stimulation in the cat

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Abstract

Functional imaging studies and clinical evidence suggest that structures in the brainstem contribute to migraine pathophysiology with a strong association between the brainstem areas, such as periaqueductal gray (PAG), and the headache phase of migraine. Stimulation of the superior sagittal sinus (SSS) in humans evokes head pain. Second-order neurons in the trigeminal nucleus that are activated by SSS stimulation can be inhibited by PAG stimulation. The present study was undertaken to identify pontine and medullary structures that respond to noxious stimulation of the superior sagittal sinus or to ventrolateral PAG stimulation. The distribution of neurons expressing the protein product (fos) of the *c-fos* immediate early gene were examined in the rostral medulla and caudal pons of the cat after (i) sham, (ii) stimulation of the superior sagittal sinus, (iii) stimulation of the superior sagittal sinus with PAG stimulation, or (iv) stimulation of the PAG alone. The structures examined for fos were the trigeminal nucleus, infratrigeminal nucleus, reticular nuclei, nucleus raphe magnus, pontine blink premotor area, and superior salivatory nucleus. Compared with all other interventions, fos expression was significantly greater in the trigeminal nucleus and superior salivatory nucleus after SSS stimulation. After PAG with SSS stimulation, on the side ipsilateral to the site of PAG stimulation, fos was significantly greater in the nucleus raphe magnus. These structures are likely to be involved in the neurobiology of migraine.

Keywords: Trigeminovascular; Migraine; Brainstem; Medulla

1. Introduction

Migraine is a phasic disorder arising from an unknown primary central nervous system dysfunction [22]. Clinical evidence and functional imaging studies suggest that the syndrome may arise from dysfunction in the brainstem. Functional imaging studies [62,63] and clinical reports [19,23,47,61] have pointed to the periaqueductal gray (PAG) and its neighboring structures as an area of potential dysfunction. Structures involved in the interaction between the trigeminovascular pathways and the PAG are, therefore, of interest in building a map of the neuroanatomy of primary headache syndromes.

Abbreviations: ITN, infratrigeminal nucleus; NHS, normal horse serum; NRGC, nucleus reticularis gigantocellularis; NRM, nucleus raphe magnus; NRMC, nucleus reticular magnocellularis; NRPC, nucleus reticularis parvocellularis; NRPGL, nucleus reticularis paragigantocellularis lateralis; PAG, periaqueductal gray; PHOSB, phosphate buffer; PB, pontine blink premotor area; PPRN, paramedian pontine reticular nucleus; SD, standard deviation; SSN, superior salivatory nucleus; SSS, superior sagittal sinus; Vc, trigeminal nucleus caudalis; vlPAG, ventrolateral PAG; VMN, trigeminal motor nucleus; VN, trigeminal nucleus; Vo, trigeminal nucleus oralis; Voc, trigeminal nucleus oralis caudalis; Vodm, trigeminal nucleus oralis dorsomedialis; Vor, trigeminal nucleus oralis rostralis; Vp, trigeminal nucleus principalis; Vpd, trigeminal nucleus principalis dorsalis; Vpv, trigeminal nucleus principalis ventralis; VST, spinal trigeminal tract

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The PAG is involved in numerous physiological functions, particularly the control of nociception and its concomitant behavioral and autonomic events [5,13,24]. The caudal ventrolateral region of the PAG (vlPAG) is responsive to activation of craniovascular afferents [28,33] and exerts a significant effect on trigeminal nociceptive neurons in the dorsal horn [36]. Electrophysiological studies show that electrical and chemical stimulation of the vlPAG inhibits trigeminal nociception [3,35,37,38,56]. The PAG exerts primarily inhibitory but also facilitatory control over dorsal horn nociceptive processing via intermediate structures in the rostral medulla and caudal pons. Of descending neurons from the PAG that project to the trigeminal nucleus, approximately 30% are direct, with the remainder being indirect projections relaying in medullary and pontine structures [7,10].

A map of brain areas activated by PAG stimulation has been made using the 2-deoxyglucose (2-DG) method [6] and other studies have investigated fos expression in the brain after stimulation of the PAG [50]. Midbrain structures expressing fos have been identified following injection of capsaicin into the cisterna magna [60] and following non-trigeminal visceral noxious stimulation [12]. However, the full detail concerning structures in the brainstem that are activated by a specific trigeminovascular nociceptive stimulus and modulated by the PAG remains to be determined. Due to the potential role for the PAG in primary headache, it is appropriate to determine which structures in the medulla and pons are potentially involved in PAG-mediated modulation of trigeminovascular nociception.

The model employed in this study draws on the observation in humans that stimulation of an intracranial vessel, the superior sagittal sinus (SSS), produces head pain referred to the ophthalmic dermatome [48]. In the cat, noxious stimulation of the SSS activates neurons in the trigeminal nucleus [31] that can be modulated by stimulation of the vlPAG [35]. This study compares neuronal activation measured by fos expression in selected structures of the pons and medulla after sham, SSS stimulation, PAG stimulation, and PAG with SSS stimulation in the cat.

2. Materials and methods

2.1. Animals and surgery

Data were obtained from 24 cats (2.9 ± 0.6 kg). Each procedure was performed in accordance with the UK Animals (Scientific Procedures) Act 1986 and adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain [65]. Cats were anesthetized with α-chloralose, 60 mg/kg intraperitoneal, then by 20 mg/kg intravenous supplements [55], after induction with halothane. Cardiovascular parameters (blood pressure and heart rate) and pupillary reaction to noxious pinching of the forepaw were used to determine the need for supplementary anesthesia. A neuromuscular blocker, gallamine triethiodide (6–20 mg/kg, i.v.), was administered during stimulation of the superior sagittal sinus and periaqueductal gray. Body temperature was monitored and maintained at 37–39 °C using a rectal thermistor probe and heater blanket unit. The cat was endotracheally intubated and ventilated. To provide optimum alveolar ventilation, end-tidal CO_2 was maintained between 2% and 4% and inspired oxygen continuously monitored. Femoral arterial and venous catheter lines were inserted for continuous measurement of blood pressure and heart rate, and administration of fluids and drugs, respectively. Arterial blood gas parameters were measured at intervals throughout the experiment, and along with blood pressure and heart rate, were maintained within the normal physiological range. The animal was placed in a stereotaxic frame for further surgery. The eyes were covered with an ointment to prevent drying of the cornea.

For exposure of the superior sagittal sinus, a midline incision of the scalp was made then a circular midline craniotomy (2 cm diameter) was drilled while cooled with saline. A 3- to 8-mm section of the superior sagittal sinus was exposed then isolated on three sides from the cortical dura mater and midline falx. A rectangle of polyethylene was placed under the sinus, and then the partially isolated sinus was gently lifted onto a pair of platinum hook electrodes for stimulation. A semi-circular plastic dam was affixed to the bone surrounding the craniotomy then filled with clean liquid paraffin to prevent dehydration and for electrical insulation against the cortex. For stereotaxis involving the periaqueductal gray, the atlas of Berman [9] was used.

At the completion of the experiment, the brain was perfused and removed. For transcardiac perfusion, 1 ml of solution containing 0.5 ml heparin (1000 KIU/ml) and 0.5 ml of 1% sodium nitrite was injected into the left ventricle of the heart. The animal was then perfused transcardially with 1.5 l of 0.9% saline followed by 2 l of paraformaldehyde 4% in 0.1 M phosphate buffer (pH 7.2–7.4), then 1 l of 30% sucrose solution in phosphate buffer.

2.2. Experimental set-up and stimulation parameters

Following the preparatory surgery, a 24-h rest period was observed, during which time the animal was kept in the stereotaxic frame, depth of anesthesia was maintained with α-chloralose, and physiological parameters maintained within normal limits. The 24-h rest period after surgery is optimal for minimizing background levels of fos elicited by preparatory surgery and anesthesia [27]. The experiments were performed within the same time course and period of the day to reduce possible effects of diurnal variation, since it has been shown that adrenal steroids affect *c-fos* expression in the trigeminal nucleus [41]. Following 2 h of stimulation, a 1-h rest period was observed, followed by perfusion.