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fMRI of supraspinal areas after morphine and one week pancreatic inflammation in rats

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ABSTRACT

Abdominal pain is a major reason patients seek medical attention yet relatively little is known about neuronal pathways relaying visceral pain. We have previously characterized pathways transmitting information to the brain about visceral pain. Visceral pain arises from second order neurons in lamina X surrounding the spinal cord central canal. Some of the brain regions of interest receiving axonal terminations directly from lamina X were examined in the present study using enhanced functional magnetic resonance imaging (fMRI) before and one week after induction of a rat pancreatitis model with persistent inflammation and behavioral signs of increased nociception. Analysis of imaging data demonstrates an increase in MRI signal for all the regions of interest selected including the rostral ventromedial medulla, dorsal raphe, periaqueductal grey, medial thalamus, and central amygdala as predicted by the anatomical data, as well as increases in the lateral thalamus, cingulate/retrosplenial and parietal cortex. Occipital cortex was not activated above threshold in any condition and served as a negative control. Morphine attenuated the MRI signal, and the morphine effect was antagonized by naloxone in lower brainstem sites. These data confirm activation of these specific regions of interest known as integration sites for nociceptive information important in behavioral, affective, emotional and autonomic responses to ongoing noxious visceral activation.

Introduction

Relatively little information has been available concerning how the brain processes visceral pain information (Mayer and Gebhart, 1994). Brain imaging techniques have become invaluable tools to assess activation of supraspinal areas. Using functional magnetic resonance imaging (fMRI), for example, brain areas activated in patients with gastrointestinal disorders have been mapped (Silverman et al., 1997; Mertz et al., 2000; Bernstein et al., 2002; Bonaz et al., 2002), cortical representation of visceral and somatic sensations compared (Aziz et al., 2000b; Dunckley et al., 2005), and modulation of visceral sensations by emotions examined (Aziz et al., 2000a). In general, data available reveal specific brain sites activated regardless of the source of pain, but visceral pain evokes greater fMRI signal intensity.

In the past few years, our group has characterized pathways relaying information to supraspinal areas about noxious stimulation of visceral structures including colon (Al-Chaer et al., 1996a, 1996b, 1996c, 1998; Willis et al., 1999; Ness, 2000), duodenum (Feng et al., 1998) and pancreas (Houghton et al., 2001, 1997; Wang and Westlund, 2001). The experimental evidence supports clinical studies in which dorsal myelotomies diminish or abolish pain in patients with pelvic and abdominal cancer (Hirshberg et al., 1996; Nauta et al., 1997, 2000; Becker et al., 1999; Kim and Kwon, 2000; Gildenberg and Hirshberg, 1984). In addition to the dorsal column midline ascending pathway, our anatomical studies have traced axons ascending in ventral white matter from visceral processing centers located around the spinal cord central canal directly to other superspinal sites (Wang et al., 1999). In addition to hypothalamic sites important in autonomic control, the axonal pathways terminate in the rostral ventromedial medulla (RVM), dorsal raphe (DR), periaqueductal gray (PAG), medial and lateral thalamus, and amygdala, known sites of nociceptive integration. The present study uses MRI signal to compare these brainstem sites known to receive input from these visceral pain pathways and selected cerebral regions of interest at two time points separated by one week, before and after development of a persisting visceral inflammatory pain state. An animal model of pancreatitis mimicking the clinical condition has been developed and the accompanying persistent pain state characterized (Vera-Portocarrero et al., 2003).

Abbreviations: DBTC, dibutyltin dichloride; fMRI, functional magnetic resonance imaging.

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The time course of the model (two weeks) is particularly useful for fMRI study and assumes a constant barrage of nociceptive information of visceral origin. The study reported here compares MRI signal before and after noxious pancreatic inflammation of one week duration. Morphine attenuation of the effect and naloxone reversal of the morphine reduction during the second session demonstrates opiate receptor involvement.

In initial animal studies, fMRI was used to assess brain regions after noxious events such as sciatic nerve stimulation (Chang and Shyu, 2001), median nerve stimulation (Scanley et al., 1997), and forepaw stimulation (Hyder et al., 1994). Fewer studies examined brainstem regions in animals after noxious visceral stimulation (Willis et al., 1999; Duong et al., 1999). Other more recent studies will be discussed. To our knowledge there are no animal MRI studies imaging persistent visceral pain responses of one week duration in specific brainstem and cortical regions.

Materials and methods

Experimental animals

All procedures were approved by the University of Texas Medical Branch Animal Care and Use Committee and adhere to the guidelines for the ethical treatment of experimental animals published by the International Association for the Study of Pain. The experimental subjects were male Lewis rats weighing 150–175 g. Animals were kept two per cage until the day of the experiment when they were kept one per cage thereafter. Low soy content diet (Harlan Teklab 8626, Madison, WI) was provided, and the animals were kept under a standard 12:12 light: dark cycle (lights on at 7 am, lights off at 7 pm).

General experimental design

Male inbred Lewis rats ($n=18$; Harlan Sprague Dawley, Houston, TX), anesthetized with 1.2–1.5% isofluorane in a mixture of oxygen and nitrous oxide (30:70), were prepared for acquisition of baseline imaging data by inserting a catheter into the tail vein for contrast tracer injection. After the set of baseline images were collected and before the animals recovered from the anesthetic state, dibutyltin dichloride (DBTC, Aldrich, Milwaukee, WI) was injected to induce pancreatitis or vehicle was injected through the tail vein catheter already in place. After the injection, animals were allowed to recover for a week in their homecage with close monitoring and special care for those with developing pancreatitis. The DBTC-induced pancreatitis was maintained with Teklab diet 8626 and 10% alcohol in the drinking water as described previously (Vera-Portocarrero et al., 2003). On day 7 after injection of DBTC ($n=12$) or vehicle ($n=6$), animals underwent a second imaging acquisition series. After the acquisition of images, the rats received either morphine ($n=3$; 5 mg/kg, Paddock Laboratories, Minneapolis, MN) or saline ($n=3$) injected intravenously through the tail vein catheter. Another set of images was acquired 30 min after the morphine or saline injection. In a separate group of animals, naloxone (1 mg/kg, Endo Pharmaceuticals Inc. PA) was injected 15 min before morphine ($n=5$) or saline ($n=3$). Naloxone is a non-specific opioid receptor antagonist widely used to confirm that effects are opioid receptor-mediated (Mason, 1999).

Acquisition and analysis of imaging data

Rats were anesthetized with 1.2–1.5% isofluorane in a mixture of oxygen and nitrous oxide (30:70). During the imaging procedures, each animal was artificially ventilated using a veterinary anesthesia ventilator (Hallowell EMC, Pittsfield, MA). A cannula was placed in the tail vein for the delivery of 2 mg Fe/kg of a superparamagnetic iron oxide tracer (SPIO; Combidex Advanced Magnetics, Inc). Combidex has a long vascular residence time ($t_{1/2}=5$ h), thereby enhancing the signal intensity decrease in proportion to increased cerebral blood volume (van Bruggen et al., 1998). To obtain coronal multislice spin echo images (TR=3 S, TE=65 ms, FOV=6×6 cm, slice thickness=1.6 mm) before and after SPIO injection, a 4.7 T magnet (Varian, INOVA) was used with a 5-cm diameter surface coil tuned to 200 MHz. The rats were placed in a supine position on a Plexiglas cradle with their heads positioned in the center of the surface coil. After the cradle was locked in place inside the magnet, a sagittal image of the rat brain using a fast low-angle shot gradient-echo imaging sequence, or FLASH (Frahm et al., 1986) was acquired to define slice positions in the hemodynamic scans. The enhanced fMRI protocol consisted of two imaging regimens: 1) multislice T_2-weighted spin echo imaging (T_2WI); and 2) single slice hemodynamic bolus tracking gradient-echo imaging in which animals are injected with super-paramagnetic iron oxide. Steady state plasma volume images supplemented bolus transit experiments. Zero filling was used to increase image resolution, particularly when isolating signal intensity profiles from arteries and veins. Each fMRI protocol took about 50 min per animal. For comparison purposes, repeated-measures ANOVA was used to identify areas where signal intensity changed significantly from baseline ($p\geq 0.05$), using Newman–Keuls post-hoc comparisons.

MRI signal intensity measurements

MRI images were analyzed for areas of localized increases in signal intensity above threshold in the regions of interest. The T_2-weighted spin echo images were compared to the Paxinos rat brain atlas (Paxinos and Watson, 1982). The use of anatomical landmarks insured that the animal was placed in the correct position from acquisition to acquisition and any variability produced by removing the animals from the magnet is reduced. Registration of the coronal brain MRI slices with the brain atlas was achieved by careful comparisons with known brain neuroanatomical landmarks that are easily visible in the MRI image, including ventricles, cerebral aqueduct, and hippocampus etc. Overlays of brain regions corresponding to various regions of interest were outlined by hand for each animal on the T_2-weighted baseline images using image processing software developed in-house (Fig. 1A) and the overlay used at the subsequent one week time point. This method insured that the same regions of interests were compared between scans. The region of interest overlays were then analyzed by an observer blinded to the treatment groups. The digital image signal intensity in the MRI images was measured, where intensity was proportional to the amount of tracer in the region of interest taken as a relative circulating blood volume. In order to control for possible variations in plasma SPIO concentrations between MRI sessions, parenchymal signal intensity measurements were normalized by dividing parenchymal signal intensity by that measured in large blood vessels within the slice of analysis. The large blood vessels consisted of either the sagittal sinus or the transverse sinus.

Regions of interest

In this study, brain areas with increased signal intensity compared to baseline were overlaid on anatomical MRI setting a blood volume threshold equal to the highest parenchymal blood volume in baseline images. Those regions that rose above that threshold were mapped. Then, using the same threshold in the "activated" image, those regions of high signal intensity were overlaid on the anatomical images in order to confirm their location in the brain. These regions coincided with areas receiving axonal input from visceral processing areas in the spinal cord in our tract tracing studies (Wang et al., 1999) and higher brain regions known to process nociceptive information. The major neural structures analyzed in this study included the rostral