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The antinociceptive effect of stimulating the retrosplenial cortex in the rat tail-flick test but not in the formalin test involves the rostral anterior cingulate cortex

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ABSTRACT

The stimulation of the retrosplenial cortex (RSC) is antinociceptive in the rat tail-flick and formalin tests. The rat RSC is caudal to and send projections to the ipsilateral anterior cingulate cortex (ACC), which is also involved in pain processing. This study demonstrated that pre-treating the rostral (rACC), but not the caudal ACC with $CoCl_2$ (1 mM), or the rACC ablation increased the duration of the antinociceptive effect evoked by a 15-s period of electrical stimulation (AC, 60 Hz, 20 μA) of the RSC in the rat tail-flick. Injecting the GABA-A antagonist bicuculline (50 ng/0.25 μL), but not the GABA-B antagonist phaclofen (300 ng/0.25 μL) into the rACC also increased the duration of the stimulation-induced antinociception from the RSC. In contrast, the effects of rACC stimulation persisted after the injection of $CoCl_2$ (1 mM) into the RSC. The injection of $CoCl_2$ into the rACC did not change the nociceptive behavior of rats during phase 1 of the formalin response but reduced licking response duration during phase 2. This effect was similar in sham or stimulated animals at the RSC. We conclude that the antinociceptive effect of stimulating the RSC in the rat tail-flick test is modulated by the rACC involving GABA-A receptors in this cortex. In contrast, the antinociceptive effect of stimulating the RSC in the formalin test does not involve the rACC.

1. Introduction

Retrosplenial cortex (RSC) stimulation produces antinociception in the tail-flick and formalin tests (Reis et al., 2010) and reduces incision pain (Rossaneis et al., 2011) in rats. The RSC is consistently activated during noxious stimulation as revealed by neuroimaging studies in rats (Hess et al., 2007). In addition, regional blood flow is higher in the RSC of fibromyalgic patients compared to control patients (Wik et al., 2003). In contrast, regional blood flow in the RSC of fibromyalgic patients is reduced during externally induced acute pain (Wik et al., 2006). A micro-PET analysis of the rat brain after spinal nerve ligation revealed decreased metabolism in the RSC (Kim et al., 2014).

The rat RSC is caudal to and sends projections to the ipsilateral anterior cingulate cortex (ACC) (Vogt and Peters, 1981; Van Groen and Wiss, 2003), which is a brain region involved in pain processing (Quintero, 2013). The stimulation of the rostral ACC (rACC), which is also known as cingulum 1 (Johansen et al., 2001; Paxinos and Watson, 2005), is hyperalgesic (Calejesan et al., 2000; Ohara et al., 2005), and its neural blockade produces analgesia in rats (Vaccarino and Melzack, 1989). In addition, the rACC mediates the aversive component of pain (Johansen et al., 2001; Kim et al., 2010).

An increase in fMRI signaling in the rACC and RSC was discovered in a rat model of pancreatitis-induced abdominal pain (Westlund et al., 2009). The ACC neuronal activity increases during escape from a noxious thermal stimulus (Hutchinson et al., 1999; Koyama et al., 2001; Iwata et al., 2005), and cingulotomy has been proposed for the treatment of several types of chronic pain (Foltz and White, 1962; Fuchs et al., 2014). The rACC activity is necessary for the perception of the aversive component of inflammatory pain in rodents (Johansen et al., 2001). Enhanced transport of Mn^{2+} observed in fMRI images during noxious stimulation of a rat forepaw were found in several brain regions, including the rACC and RSC; however, only the enhancement in RSC was not attenuated by intraperitoneal morphine (Yang et al., 2011). These findings led to the notion that antinociception evoked by RSC stimulation is not due to spreading to the rACC. However, rACC involvement in the antinociceptive effect of RSC stimulation has yet to be conducted.

The present study evaluates whether rACC modulates the stimulation-induced antinociception from the RSC. Changes produced by injecting $CoCl_2$ into or surgical ablation of the rACC against the antinociceptive effect of RSC stimulation was evaluated using the tail-flick and formalin tests in rats. Similar experiments were conducted by injecting GABA-A (bicuculline) or GABA-B (phaclophen) receptor antagonists into the

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rACC because GABA receptors were already demonstrated in this region (Mengod et al., 1990; Bozkurt et al., 2005; Luna-Munguía et al., 2005; Rivera et al., 2008; Palomero-Gallagher et al., 2009). Because a caudal portion of the ACC (cACC) exists between the RSC and the rACC, we also examined whether injecting $CoCl_2$ into the cACC changes the stimulation-induced antinociception from the RSC in the rat tail-flick test.

2. Materials and methods

2.1. Subjects and surgery

Male Wistar rats (140–160 g) were housed two to a cage with free access to food and water and maintained at a controlled temperature (23 ± 1 °C) with a 12-h light–dark cycle before and after surgery (light cycle beginning at p.m. 7:00 h). The experiments were approved by the Commission of Ethics in Animal Research, Faculty of Medicine of Ribeirão Preto, University of São Paulo (Protocol number 240/2005). The proposals of the Committee for Research and Ethical Issues of IASP (Zimmermann, 1983) were followed throughout the experiments.

Each animal was anesthetized with tribromoethanol (250 mg/kg, i.p.), and a Teflon-insulated monopolar electrode (o.d. = 0.125 mm) was stereotaxically implanted into the skull to lie in the left RSC using the coordinates (in mm) AP = 5.0 (from ear bars); L = 1.0 (from the midline) and H = −1.9 (from the skull surface). A 12-mm length of a 23-gauge stainless steel guide cannula was implanted stereotaxically into the skull until its tip was 0.5 mm above the left rACC using the coordinates (in mm) AP = 9.2; L = 1.0, and H = −2.0.

A 12-mm length of a 23-gauge stainless steel guide cannula was implanted stereotaxically into the skull until its tip was 0.5 mm above the left rACC using the coordinates (in mm) AP = 9.2; L = 1.0, and H = −2.0, or 0.5 mm above the left cACC using the coordinates (in mm) AP = 8.0; L = 1.0, and H = −2.0.

The electrode and guide cannula were fixed to the skull with two screws and dental cement. One screw was used as the reference electrode. The guide cannula was kept patent with a sterile obturator until the time of drug administration. Animals were then given penicillin (50 mg/kg, i.m.) and allowed to recover for at least one week before the experiment.

Cortical ablations were performed under anesthesia with tribromoethanol (250 mg/kg, i.p.). Animals were placed in a stereotaxic frame and an electrode was implanted into the left RSC. A window including the rACC was then opened in the skull and the rACC was removed by gentle aspiration as previously validated (Lamas et al., 2013). Sham ablated rats also had a window opened in the skull but no aspiration was performed. Rats were given penicillin (50 mg/kg, i.m.) after the ablations and allowed to recover for at least one week before the experiment.

2.2. Tail-flick test

Each animal was placed in a ventilated tube with the tail laid across a wire coil maintained at room temperature (23 ± 2 °C). The coil temperature was then increased by the passage of an electric current, and the latency for the tail withdrawal reflex was measured. Heat was applied to a portion of the ventral surface of the tail between 4 and 6 cm from the tip. Tail-flick latency (TFL) was measured in 5-min intervals until a stable baseline was obtained over three or four consecutive trials. The apparatus was fixed to obtain a baseline TFL at approximately 3 (for rACC experiments) or 9 (for cACC experiments) seconds. Only rats showing stable baseline TFL after up to 6 trials were used in each experiment. Each trial was terminated after 6 (for RSC stimulation) or 15 (for ACC stimulation) seconds to minimize the possibility of skin damage.

2.3. Formalin test

Rats pre-treated with $CoCl_2$ or saline in the rACC were stimulated at the RSC for 15 s with a current intensity of 20 μA. Soon after this procedure, 5% formalin (50 μL) was injected subcutaneously into the dorsal surface of a hind paw with a 25-gauge needle. The hind paw contralateral to the cortical targets was chosen according to Reis et al. (2012). The number of spontaneous injected paw flinches, and the total time in which the animal licked the injected paw were counted during the 5 min of post-injection period (phase 1 of the response to formalin). Separated groups of rats were stimulated at the RSC, 10 min after formalin. The number of spontaneous injected paw flinching, and the total time in which the animal licked the injected paw were counted every 5-min for up to 60 min after formalin (phase 2 of the response to formalin). Flinching was characterized as a rapid and brief withdrawal or flexion of the injected paw.

2.4. Stimulation procedures

Twenty minutes after the intracerebral injection, electrical stimulation (AC, 60 Hz) was applied during 15 s to the cortical target at an intensity of 20 μA as suggested elsewhere (Reis et al., 2010). During the stimulation period, rats were gently restrained by hand, and the drop in voltage across a 1-kΩ resistor in series with the electrode was continuously monitored on an oscilloscope. The TFL was recorded 30 s after cortical stimulation and then at a 5-min interval for up to 30 min. No attempt was made to test for the presence of antinociception during stimulation. Sham stimulated rats (control) had identical electrode implant procedures and connections to the stimulator assembly. They also received either saline or a drug into the rACC or cACC but no current was passed through the electrode.

2.5. Intracerebral injection

A drug or vehicle was microinjected into the rACC or cACC, or RSC using a glass needle (70–90 μm, o.d.) protected by an assembly of telescoping steel tubes as proposed elsewhere (Azami et al., 1980). The assembly was inserted into the guide cannula and the needle advanced to protrude 1.0 mm beyond the guide cannula tip. The volume of microinjection was 0.25 μL, delivered at a constant rate over a period of 3 min. The needle was removed 20 s after completion of the injection.

2.6. Histology

At the end of the experiments, rats were deeply anesthetized with intraperitoneal sodium thiopental (60 mg/kg) and perfused through the heart with 4% paraformaldehyde in 0.1 M phosphate buffered saline. Fast green (0.5 μL) was injected through the guide cannula to label the site of intracerebral injection. The brain was removed and the electrode track or dye spot localized from 50-μm serial coronal sections was stained with neutral red and identified on diagrams from the atlas of Paxinos and Watson (2005). Only animals that had the electrode or dye spot position confirmed by histology were considered for data analysis.

2.7. Experimental design

2.7.1. Effects of neural block of rACC or cACC on the stimulation-produced antinociception from the RSC in tail-flick test

Four groups of 8 rats each had an electrode implanted into the rACC and a guide cannula into the RSC. Another four groups of 8 rats each had an electrode implanted into the cACC and a guide cannula into the RSC. Saline (0.25 μL) or 1 mM $CoCl_2$ (0.25 μL) was injected into the rACC or cACC soon after recording of the baseline TFL. Electrical or sham stimulation of the RSC was performed 20 min later, and TFL was recorded within 30 s after the end of the stimulation period and then for up to 30 min at 5-min intervals. Saline/sham stimulated rats were taken as control.