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A novel method for subarachnoid drug delivery in the medullary region of rats

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Abstract

This study describes a novel method for direct subarachnoid drug delivery to the medullary dorsal horn region of rats, without introducing a catheter. The reliability of the method was demonstrated by a pharmacological validation; that is, morphine administration to the medullary region blocked the nociceptive response to formalin injected in the temporomandibular joint (TMJ) region, an effect that was prevented by co-administration of naloxone.

The method proposed offers many advantages over the existing methods for medullary drug delivery with catheter implantation. It is easy to be employed, it does not induce any sign of motor impairment, and it does not require the neck surgery performed to implant a catheter in the medullary dorsal horn region. Therefore, it is a useful method for subarachnoid drug delivery in behavioral trigeminal pain studies, particularly when nociceptive behavioral measures that require normal neck muscle activity to occur, such as head withdraw or head flinch are evaluated.

Keywords: Subarachnoid drug delivery; Orofacial; Nociceptive behavior; Medullary dorsal horn

1. Introduction

The orofacial region is one of the most densely innervated areas of the body, which focuses common acute, chronic and referred pain (Sessle, 2000). Drug administration to the medullary cerebrospinal fluid is a useful tool in the orofacial pain research, and is currently accomplished by a catheter implantation in the surroundings of trigeminal subnucleus caudalis, also known as medullary dorsal horn. This procedure is commonly performed through a surgical exposition of the dorsal surface of the neck and insertion of a catheter in the subarachnoid space through a slit in the atlanto-occipital membrane (Aigouy et al., 1992; Flores et al., 2001; Tambeli et al., 2001; Wang et al., 2002).

It is well known that the ability to correlate a behavioral measure with pain arising from an orofacial region in animal studies is essential in elucidating the underlying mechanisms of pathophysiology of orofacial pain syndromes and temporomandibular disorders. However, the surgical catheter implantation performed to study the effect of drugs delivered in the medullary dorsal horn region may affect some of the frequently used nociceptive behavioral measures that require normal neck muscle activity to occur, such as head withdraw in response to a local mechanical stimulus (Anderson and Rao, 2001; Christensen et al., 2001; Imbe et al., 2001; Benoliel et al., 2002b; Ogawa et al., 2003), or head flinch induced by local chemical stimulation (Anderson and Rao, 2001; Roveroni et al., 2001; Chidiac et al., 2002; Gameiro et al., 2003; Hartwig et al., 2003; Clemente et al., 2004). Therefore, the aim of this study was to develop a method for direct subarachnoid drug delivery to the medullary region that facilitates animal investigation of orofacial pain, and that can be combined with many orofacial pain models, particularly with those that use nociceptive behavior measures, such as head flinch or head withdraw.

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2. Methods

2.1. Animals

Experiments were performed on 250–320 g male Wistar rats housed (five per cage) in a temperature-controlled room (23 ± 1 °C) on a 12-h light:12-h dark cycle (lights on at 6 a.m.), with food and water available ad libitum. Animals were handled for at least 1 week prior to the experiments. Experimental protocols were approved by the Committee on Animal Research of the University of Campinas and conformed to IASP guidelines for the study of pain in animals (Zimmermann, 1983).

2.2. General procedures

Testing sessions took place during the light phase in a quiet room maintained at 23 °C. Prior to the experiments, each animal was placed in the test chamber (30 cm × 30 cm × 30 cm mirrored-wood chamber with a glass at the front side) for a 15-min habituation period.

2.3. Drugs

Formalin solutions were prepared from commercially (Sigma) available stock formalin (an aqueous solution of 37% of formaldehyde) further diluted in 0.9% NaCl (saline) to concentration of 1.5% (Roveroni et al., 2001). Morphine sulfate (Sigma) 3, 6 and 9 μg (Grabow and Dougherty, 2001) and naloxone hydrocloride (Sigma) 15 μg (Danzebrink et al., 1995) were dissolved in saline.

2.4. Subarachnoid medullary injection

Rats were briefly anesthetized with halothane, and a small area of skin overlying the high cervical region was shaved with an electric razor. Animals were dorsally positioned, so that the sub occipital space could be easily found.

A 30-gauge needle connected to a 50-μl Hamilton syringe by a polyethylene cannula was first inserted below the occipital bone up to 4 mm, and slightly inclined in a cranial direction. The needle was advanced more than 2 mm to perforate the atlanto-occipital membrane and reach the medullary subarachnoid space (Fig. 1). This technique allowed direct drug delivery in the cerebrospinal fluid in the surroundings of trigeminal subnucleus caudalis. Total injection volume in all experiments was 10 μl. All injections were performed at rate of 1 μl/s. Each animal regained consciousness approximately 30 s after discontinuing the anesthesia.

2.5. Testing for correct site of subarachnoid injection

In preliminary experiments, the injection procedure was tested by the administration of Evans blue dye (1%, 10 μl) in 10 rats. Following the injection, the rats were euthanized by a lethal dose of halothane. Cervical laminectomy and occipital craniotomy were performed using blunt dissection techniques and the site of injection as well as dye spread was examined.

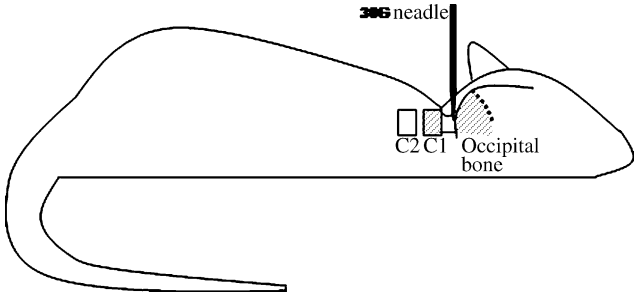

Fig. 1. The approach used for the subarachnoid drug injection. The needle was inserted below the occipital bone up to 4 mm, and slightly inclined in a cranial direction. The needle was advanced more than 2 mm to perforate the atlanto-occipital membrane and reach the medullary subarachnoid space.

2.6. Testing procedure for temporomandibular joint pain

Animals were briefly anesthetized by halothane inhalation to allow the temporomandibular joint (TMJ) injection, which was performed with a 30-gauge needle connected to a 50-μl Hamilton syringe. Injection volumes were 50 μl in all cases. Each animal regained consciousness approximately 30 s after discontinuing the anesthesia and was returned to the test chamber for counting nociceptive responses during a 45-min observation period. The nociceptive response score was defined as the cumulative total number of seconds that the animal spent rubbing the orofacial region asymmetrically, with the ipsilateral fore or hind paw, plus the number of head flinches counted during the observation period, as previously described (Roveroni et al., 2001). From a theoretical perspective, the occurrence of a given behavior is expressed as the proportion of time that the behavior occupies. Since head flinches followed a uniform pattern of 1 s of duration, each flinch was expressed as 1 s (Roveroni et al., 2001). At the conclusion of the experiments, rats were anesthetized by inhalation of 4% halothane and maintained at halothane level of 1.5–2%. Evans blue dye (1%, 5 mg/kg) was then intravenously administered in order to visualize formalin-induced plasma extravasation. Ten minutes later, the animals were euthanized by halothane inhalation and a *post-mortem* examination of the injected TMJs was performed (Haas et al., 1992). This procedure also allowed confirmation that the plasma extravasation induced by the TMJ injection at the correct site was restricted to the immediate TMJ region.

2.7. Motor assessment

To verify whether the subarachnoid injection in the medullary region induces motor impairment, an extra set of experiments was performed using the rota-rod test. Rats were initially trained at a low velocity and the cut-off time was 120 s. After a subarachnoid injection of either saline of