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SCIENCE DIRECT
Neuropharmacology 50 (2006) 769–776
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

The locus coeruleus nucleus as a site of action of the antinociceptive and behavioral effects of the nicotinic receptor agonist, epibatidine☆

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Received 6 June 2005; received in revised form 25 October 2005; accepted 27 November 2005

Abstract

The mechanisms and sites of action of epibatidine-induced antinociception and side effects are poorly understood. The present study tested the hypothesis that the locus coeruleus is a site of action of epibatidine. Behavioral responses of rats to hindpaw formalin injection were compared after direct administration of epibatidine into the locus coeruleus (LC), and after subcutaneous administration. Different groups of rats were injected with formalin into the rear paw after administration of either ACSF, epibatidine (0.01, 0.06, 0.12, and 0.3 μg) into the locus coeruleus or epibatidine (2.5–5 μg/kg) subcutaneously. Assessment of pain-related behavior was done by evaluating the incidence of favoring, lifting and licking of the injected paw in the different groups. Abnormal motor behavior was also recorded. Infusion of epibatidine into LC induced analgesia, which was reversed by prior infusion of mecamylamine into LC. Epibatidine into the locus coeruleus resulted in a significant lower pain score in the second phase of the formalin test compared to control rats and was as effective as subcutaneous epibatidine. The analgesic effects of epibatidine were regionally selective in that the administration of epibatidine outside the locus coeruleus area was not analgesic. The every tested dose of epibatidine administered into the locus coeruleus also produced freezing behavior immediately after injection, which was relatively short-lived compared to the analgesic effect. Freezing was inhibited by administration of mecamylamine into the LC. Together the results implicate the LC as a target for the analgesic effects of epibatidine.

Keywords: Epibatidine; Analgesia; Locus coeruleus; Pain; Behavior

1. Introduction

Nicotine and nicotinic agonists have been known for many years to have analgesic properties. However, the high incidence and severity of side effects associated with these drugs have limited their clinical use. Recent studies on the analgesic effects of epibatidine, a nicotinic acetylcholine receptor (nAChR) ligand (Bannon et al., 1998; Qian et al., 1993; Sullivan et al., 1994), and other epibatidine derivatives such as ABT-594 (Bannon et al., 1998) have triggered a new interest on the mechanism of antinociception produced by nicotinic agonists. It has been postulated that nicotinic acetylcholine receptor agonists produce their antinociceptive effects predominantly via activation of descending inhibitory pain pathways originating in the brainstem regions including the nucleus raphe magnus (Bitner et al., 1998).

Central modulation of pain involves the nucleus raphe magnus (NRM), dorsal raphe (DR) and locus coeruleus (LC). The NRM can directly control pain transmission in the dorsal horn of the spinal cord via descending projections. The effects of the DR on the spinal cord are most likely mediated by its interconnection with the NRM (Wang and Nakai, 1994). The antinociceptive effects of the LC are probably mediated both by the DR and direct projections to the dorsal horn (Proudfit and Clark, 1991; Tjolsen et al., 1991). There is clear experimental evidence that the

☆ This work was supported by the Foundation for Anesthesia Education and Research.
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0028-3908/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.neuropharm.2005.11.026

NRM (Bitner et al., 1998; Curzon et al., 1998) and DR (Cucchiaro et al., 2005) mediate the antinociception produced by epibatidine and the nicotinic agonist ABT-594. It is unknown whether the LC can also produce analgesia when stimulated with nicotinic agonists. There are several experimental evidences suggesting that the LC may respond to nicotinic agonists and induce analgesia. Electric stimulation (West et al., 1993) and morphine application (Pan et al., 2004) on the LC have antinociceptive effects. The largest collection of noradrenergic neurons resides in the pontine LC (Grimm et al., 2004) and these neurons express nAChR containing the alpha3,4,5,7 and beta2,3 subunits (Cucchiaro and Commons, 2003; Vincler and Eisenach, 2003) which are thought to be a primary receptor site for epibatidine. There are multiple data showing that the systemic administration of nicotine modifies neural activity in the LC (Erhardt et al., 2000; Kawahara et al., 1999), with two different types of excitation, short and long lasting, depending on the dose administered (Engberg and Hajos, 1994). Nicotine can induce a concentration dependent release of norepinephrine in LC cells in culture as well as in the LC of conscious animals (Gallardo and Leslie, 1998; Van Gaalen et al., 1997). Together these findings suggest that the LC could be an important contributor to the positive effects of nicotinic ligands on antinociception. However, there are no data on the interplay between noradrenergic neurons localized in the LC, nicotine agonists and antinociception.

The aim of the present study was to test if the LC is a target for epibatidine-induced antinociception or side effects. The effect of local administration of epibatidine into the LC on nociceptive response and motor behavior was measured. These data have been then compared with those observed in rats that received systemic epibatidine.

2. Methods

Male Sprague–Dawley rats (250–300 g) were housed in pairs under a 12:12-h light/dark cycle with water and food available ad libitum. For all experiments that used implanted cannulas, rats were singly housed. The protocols were in accordance with the animal care guidelines at the University of Pennsylvania and The Children's Hospital of Philadelphia and followed the Guide for the care and use of laboratory animals as adopted and promulgated by the U.S. National Institute of Health.

2.1. Surgical procedure

Rats were anesthetized with halothane and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the skull on a horizontal plane. A hole was drilled to accept a skull screw. Coordinates for the placement of the intracranial cannula guides were from intra-aural zero: antero-posterior −0.31 mm; mediolateral +0.11 mm; and dorso-ventral −0.51 mm. The entry angle was 0 degrees from the vertical. Cannula guides (26 gauge, Plastics One Inc., Wallingford, CT) were positioned and cranioplastic cement was used to affix the cannula guide to the skull and skull screw. A dummy cannula was inserted into the guide to keep it clear. Rats were allowed to recover 3 days prior to the behavioral studies.

2.2. Peripheral epibatidine injection

Three groups of rats received subcutaneous saline (control group, $n = 9$), epibatidine 2.5 μg/kg ($n = 9$) or epibatidine 5 μg/kg ($n = 9$). The study drugs were injected into the back of the rats, in the lumbar area. Formalin 5% (50 μl) was then injected subcutaneously into the plantar surface of one rear paw, using a 27-ga needle and an insulin syringe. This group of rats was not implanted with intracranial cannulas.

2.3. Intra-LC infusions

2.3.1. Epibatidine injection

Rats were implanted with an LC cannula guide. Rats were infused with either ACSF, or epibatidine at different doses: 0.01, 0.06, 0.12, and 0.3 μg in 300 nl ACSF. To verify that nAChR alone was responsible for the effects observed after the infusion of epibatidine into the LC, the nAChR channel blocker mecamylamine (1 μg) was infused into the LC 10 min prior to the infusion of 0.015 μg epibatidine in a separate group of rats.

Infusions were done by replacing the dummy cannula with an internal cannula (33 gauge) connected to a syringe by PE tubing. The drugs were injected via a syringe pump (Model 11 plus, Harvard Apparatus Inc., Holliston, MA) over 1 min. At the end of the intracranial infusion of ACSF or epibatidine, formalin 5% (50 μl) was injected subcutaneously into the plantar surface of one rear paw, using a 27-ga needle and insulin syringe.

2.3.2. Mecamylamine injection

Twelve rats were implanted with an LC cannula guide. Rats were infused into the LC with mecamylamine (1 μg) 10 min prior to the subcutaneous administration of 2.5 μg/kg epibatidine. At the end of the systemic administration of epibatidine, formalin 5% (50 μl) was injected subcutaneously into the plantar surface of one rear paw.

2.4. Behavioral assessment

To habituate them to the formalin test environment, rats were singly placed in the test chamber for 3 days for 10–15 min. The testing room was maintained at 22 °C, under normal lighting conditions. The formalin test was carried out in a 60 × 30 × 40 clear glass chamber with a mirror under the floor to allow a complete view of the animal and paws. After an initial 20-min baseline recording, rats were injected with ACSF or epibatidine via the LC cannula. The injections were made using a syringe pump, Model 11 plus (Harvard Apparatus Inc., Holliston, MA). The volume used was the same in each experiment, 300 nl, and it was infused over 1 min. Rats were videotaped during the behavioral experiments for later scoring. To score, behavior was rated for 60 min after the formalin injection. Using a time-sampling method, rats were scored every 20 s for pain behavior using four mutually exclusive categories of behavior (Abbott et al., 1999): (1) normal behavior (equal weight bearing on both hindpaws); (2) favoring (injected paw resting on the floor without pressure on the footpad); (3) lifting (injected paw elevated without touching the floor) and (4) licking (injected paw licked or bitten).

The observer who evaluated the rats' behavior was not blinded to the type of drug infused or concentration used. However, the evaluation was done before the histological confirmation of the correct placement of the cannulas and the observer did not know whether the study drug was correctly infused into or outside the LC at the time of the behavioral evaluation.

Preliminary observations suggested that epibatidine locally administered into the LC influences motor behavior, therefore offset 20-s intervals and independent from pain behavioral categories, and locomotor behavior was also scored using time-sampling method. Behavior was scored as: (1) normal locomotor, grooming and exploratory behavior, including relaxed stationary postures with natural head and limb movements; (2) freezing, characterized by complete immobility of all limbs and paws, minimal movement of the head, eyes are open and staring at a specific point with preserved muscle tone (Chung et al., 2000).

2.5. Histology

At the end of the experiment, rats were anesthetized with intraperitoneal pentobarbital (50 mg/kg) and perfused via the ascending aorta with saline for 2 min followed by a 5-min perfusion with 4% paraformaldehyde in