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Research article

The non-peptide CRH1-antagonist CP-154,526 elicits a paradoxical route-dependent activation of the HPA axis

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HIGHLIGHTS

- The non-peptide CRH1-antagonist CP-154,526 (CP) was administered to rats intraperitoneally or intraarterially.
- Intraarterial administration suppressed levels of ACTH induced by stress, while intraperitoneal administration did not.
- Intraperitoneal, but not intraarterial injection of CP dramatically increased ACTH levels in the blood by itself.
- Intraperitoneal injection of CP increased blood pressure and expression of c-fos in paraventricular hypothalamic nucleus.

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ABSTRACT

The corticotropin-releasing hormone (CRH) plays an important role in mediating physiological response to stress and is thought to be involved in the development of various psychiatric disorders. In this paper, we compare the differences between the effect of intraperitoneal (i.p.) and intraarterial (i.a.) administration of the non-peptide CRH_1 antagonist CP-154,526 (CP) (10 and 20 mg/kg) on plasma adrenocorticotropic hormone levels (ACTH), heart rate, MAP, and c-Fos expression in the paraventricular nucleus of the hypothalamus. Intraperitoneal, but not i.a., injection of CP resulted in an increase in plasma ACTH (from 105 ± 13 to 278 ± 51 pg/ml after 20 mg/kg). This effect was accompanied by a dramatic increase in c-Fos expression in cells immunoreactive for CRH in the paraventricular nucleus of the hypothalamus. When the drug was administered i.p., CP-induced activation of the HPA appears to mask the inhibitory effect of CP on stress-induced ACTH secretion, an effect which was readily apparent when the drug was given i.a. Intraperitoneal administration of CP also increased the baseline MAP which may account for previous reports that treatment with this drug attenuated the increases associated with stress. CP given by either route had no effect on baseline heart rate or stress-induced tachycardia. Thus, in all studies in which CP 154,526 is given, the route of delivery must be given careful consideration.

1. Introduction

Dysregulation of central corticotropin-releasing hormone (CRH)-dependent mechanisms is thought to play a role in the development, and course of depression and anxiety [1,10]. CRH receptor antagonists, and, specifically, CRH_1-receptor antagonists, were considered a promising class of antidepressants and anxiolytics [3,4,14,28,30,34]. Since peptide-based CRH antagonists such as alpha-helical CRH_{9-41} poorly penetrate the blood-brain barrier, they have little effect on central CRH receptors except when administered intracerebroventricularly (i.c.v.) [19]. Non-peptide selective CRH antagonists, which are capable of crossing the blood-brain barrier, can block CRH receptors in the brain when given systemically, and therefore may be clinically useful [10].

Abbreviations: ACTH, adrenocorticotropic hormone; CP, CP-154,526 {butyl[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-*d*]pyrimidin-4-yl]ethylamine}; CRH, corticotrophin-releasing hormone; HPA axis, hypothalamo-pituitary-adrenal axis; HR, heart rate; i.a., intraarterial(ly); i.c.v., intracerbroventricular(ly); i.p., intraperitoneal(ly); i.v., intravascular(ly); LPS, lipopolysaccharide; MAP, mean arterial pressure; MBP, mean blood pressure; PBS, phosphate-buffered saline; PVN, paraventricular nucleus of hypothalamus; RIA, radioimmunoassay; TBS, tris-buffered saline.

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Two non-peptide CRH_1-receptor antagonists with a pyrrolopyrimidine structure have been described: CP-154,526 (CP) [27] and antalarmin [32]. Both antagonists have been shown to inhibit CRH-induced adrenocorticotropic hormone (ACTH) release [16,27,32] and to ameliorate anxiety and depression in animal models [5,7,16,17].

CRH and its receptors are involved in the neuroendocrine and behavioral responses to stress [18,23,34]. Nalivaiko and Blessing demonstrated that CP inhibits tachycardia and cutaneous vasoconstriction in reaction to a variety of stressors in rabbits [20,21]. The original aim of our work was to investigate the effects of CP on the response of the cardiovascular and neuroendocrine systems to a strong emotional stressor in conscious rats.

Unexpectedly, we have found that CP caused a route-dependent activation of the hypothalamo-pituitary-adrenal (HPA) axis as indicated by plasma levels of ACTH and c-Fos expression in the paraventricular nucleus of hypothalamus (PVN). Furthermore, we learned that this activation completely masked any suppression that CP had on stress-evoked HPA activation.

2. Experimental procedures

2.1. Animal model

Male Sprague-Dawley rats (250–300 g) were used for all experiments. The animals were individually housed under standard controlled conditions (lights on 0700-1900, room temperature of 23–25 °C) with free access to food and water. All procedures described were approved by the Indiana University Institutional Animal Care and Use Committee and followed NIH guidelines.

2.2. Surgical procedures

All rats received two separate surgical procedures. First, telemetric transmitters (TA11PA-C40, Transoma Med, St.Paul, MN) were implanted under pentobarbital anesthesia (50 mg/kg, i.p.) as previously described [29]. After at least seven days of recovery, rats were anesthetized with ketamine/xylazine (80 mg/kg ketamine. 12 mg/kg xylazine, i.p.) and catheters were placed in a femoral artery for blood sampling and drug administration [29] and flushed daily with heparin. The catheter was tunneled subcutaneously, exteriorized, and fixed at the nape of the neck with a small leather harness.

Experiments were initiated not earlier than 7 days after the second surgery. All animals for which data are reported remained in good health throughout the course of surgical procedures and experimental protocols as assessed by appearance, behavior and maintenance of body weight.

2.3. Drugs

Chemicals were obtained from the Sigma-Aldrich (St. Louis, MO) unless specified otherwise. CP-154,526 was a gift of Pfizer Inc. (Groton, CA). First, CP was dissolved in DMSO under sonication, then Tween-80 was added, and then this was diluted with saline so that the final mixture was DMSO:Tween-80:Saline (5:5:90 by volume).

2.4. Air-jet stress

Each rat was placed in a narrow plastic restraining tube, and air was blown into the animal's face at a rate of 40 l/min for 10 min as described previously [29]. Blood was drawn via the arterial catheter just before the air-jet was turned off and the animal was removed from the tube. Following air-jet stress and blood sampling, each animal was returned to its home cage.

2.5. Experimental protocols

All experiments were performed between 10:00 a.m. and 2:00 p.m. to avoid the effect of circadian variability on plasma levels of ACTH.

The first two experimental series were performed in rats with telemetric probes and arterial catheters. Two doses of CP (10 or 20 mg/kg, in a volume of 1 ml/kg) or vehicle were given i.a. (series 1, n = 4) or i.p. (series 2, n = 5) 30 min prior to air-jet stress. Within series, each animal received three treatments (two doses of CP or vehicle) in random order allowing two days between treatments. Blood samples were drawn 5 min before CP was delivered, 25 min after, and at the 10th min of air-jet stress (i.e., 40 min after rats were given CP).

To investigate the possibility that the increases in ACTH seen with i.p administration of CP were secondary to pain from the acidity of the solution (pH ~2.0), a third group of animals, instrumented with arterial catheters (n = 3), had their CP (20 mg/kg) or equivalent volume of vehicle delivered i.p, with the pH of the injectate adjusted to 7.4. Each rat received two treatments (CP vs vehicle) in random order with two days between treatments. Blood samples were drawn at 5 min before and at 25 and 40 min after injection to match the time-course of blood withdrawals in series 1 and 2, but without any additional interventions.

In series 4, rats were given an i.p. dose of either 20 mg/kg of CP or vehicle. Ninety minutes later, animals were deeply anesthetized with pentobarbital (80 mg/kg, i.p.) and immediately perfused transcardially with 60 ml of cold saline (4 °C) followed by 60 ml 4% paraformaldehyde. Brains were then removed and postfixed in 4% paraformaldehyde for 2 h, saturated with 30% sucrose, and frozen in dry ice until further processing.

2.6. Blood sampling and analysis

Blood samples (0.35 ml) were collected in syringes containing 60 μl of a solution of 10 mg/ml EDTA and 50% aprotinin in saline. Withdrawn blood was replaced with sterile saline. Blood was immediately centrifuged at 4 °C, and the plasma was removed and stored at −80 °C until analyzed for ACTH content by radioimmunoassay (RIA) [15]. Samples were diluted to be in the linear part of the calibration curve (1–10 pg ACTH/tube) and run in duplicate [12].

2.7. Light-microscopic double-labeling immunocytochemistry of Fos-immunoreactive nuclei and CRH neurons in the PVN

To determine the extent of c-Fos activation in the nucleus of cells within the parvocellular division of the PVN after injection of CP or vehicle, 25–μm coronal sections were cut with a cryostat (Cryocut model 1800, Reichert-Jung) through the PVN and collected in PBS (pH 7.4). The sections were washed in PBS, treated with 1% sodium borohydride in deionized water for 30 min, and then rinsed several times in deionized water and PBS until the sections were free of bubbles. The sections were then placed in 0.5% H_2O_2 in PBS for 15 min to remove the endogenous peroxidase activity from the tissues, washed in PBS, and treated in 0.5% Triton X-100 in PBS for 30 min to improve antibody penetration. Following preincubation in 10% normal horse serum for 25 min, the sections were incubated in the rabbit antiserum against c-Fos (Ab-5, Calbiochem, San Diego, CA) diluted 1:12,000 for three days at 4 °C with continuous agitation on a rotary shaker. All primary antisera dilutions were made in 1% normal horse serum in PBS containing 0.08% sodium azide and 0.2% Kodak Photo-Flo (Rochester, NY). After thorough rinsing in PBS, sections were incubated in biotinylated donkey anti-rabbit IgG (1:200, Jackson Immunoresearch Laboratories, West Grove, PA) for 2 h. The sections were then washed three times in PBS and incubated in an