Short communication

Antalarmin blockade of corticotropin releasing hormone-induced hypertension in rats

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Abstract

Central administration of CRH results in endocrinological, cardiovascular, and behavioral effects that suggest stress or anxiety. Among these is a marked pressor response. Parenteral administration of CRH, however, results in hypotension. We used parenteral administration of antalarmin, a novel, small molecule CRH1 receptor antagonist, and α-helical CRH, a peptidic CRHR1/CRHR2 antagonist to attempt to determine the receptor mechanisms through which CRH is acting in both of these situations. Our results suggest that the hypertension produced by central CRH administration is mediated through central CRHR1 receptors, whereas the hypotension produced by parenteral CRH administration is mediated through peripheral CRHR2 receptors.

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Corticotropin releasing hormone (CRH) has come to be regarded as one of the most critical integrative elements in the multifaceted response to stress, playing a role in initiation, modulation, and inhibition of this response, as well as maintaining the normal rhythms of the endocrinological system that are involved in stress. The role of CRH in the behavioral, endocrinological, and physiological effects of stress has been expanding rapidly since it was identified by Vale and colleagues in 1981 [13]. Of particular interest is the role of CRH in stress-related diseases including such diverse conditions as depression, drug abuse, inflammation, eating disorders, and hypertension [11]. The possibility that CRH antagonists could be effective in treating any of these diseases has attracted the interest of a number of investigators.

CRH has interesting effects on blood pressure in rodents. When given intracerebroventricularly (i.c.v.), it produces a marked hypertension in rats [9], probably through an action on central CRH receptors. When it is given intravenously, it produces a hypotensive effect [3], which, because of the limited access of parenterally administered CRH to the central nervous system, is probably through an action on peripheral CRH receptors. However, it is not known which of the subtypes of CRH receptors might mediate the changes in blood pressure produced by CRH administration.

Three CRH receptors have been identified in rats and in humans: CRHR1, CRHR2\textsubscript{α} and a splice variant, CRHR2\textsubscript{β} [8,12]. The CRH1 and CRH2 receptors have similar structures and G-protein coupled mechanisms of action [4,11], but markedly different anatomical locations in the brain [1]. The CRH1 receptor is thought to be the most likely mediator of the endocrinological and behavioral responses to stress [2,6]. The role of the CRH2 receptor is less clearly defined, but its location on arterioles in the brain suggests a possible role in regulating blood pressure.

Antalarmin is one recently developed small molecule CRH antagonist which has been reported to have high...
affinity for the CRH1 receptor. Antalarmin and a close structural relative, CP-154,526, have been shown to block CRH-induced increases in ACTH [10,14]. In some cases these small molecule antagonists have been shown to block some of the anxiety-like effects of administration of CRH [10], but this interaction is not always clear-cut [5]. Although the peptide antagonist α-helical CRH9-41, has been shown to block the hypertensive effects of parenteral administration of CRH [3], no studies of the ability of antalarmin to modify the blood pressure effects of CRH administration have been reported. Thus, it is not known whether the hypertensive effect of parenteral CRH is mediated through an action on CRH or CRH2 receptors (but see Turnbull & Rivier, 1997 [11]; they suggest a CRH2 receptor mechanism), or whether the hypertensive effect of central CRH is through an action on either type of CRH receptor.

The current study utilized antalarmin, which produces a selective blockade of CRHR1-receptors, and α-helical CRH9-41, which has affinity for both receptors, but does not readily cross the blood–brain barrier, to characterize the diverse effects of CRH on blood pressure in awake, freely moving rats. Male Wistar rats (Harlan) were used as subjects in this study. Prior to surgery, the rats were group-housed in standard plastic cages in a vivarium that was accredited by the American Association for Accreditation of Laboratory Animal Care. The room was maintained at 20±2°C on a 12 h light/dark cycle (lights on at 7:00 a.m.). The rats had ad-libitum access to food and water throughout the study. Following surgery, the rats were singly housed in plastic cages until the termination of the study.

A lateral ventricular cannula for i.c.v. injections was implanted in the rats following anesthesia with separate injections of ketamine (100 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.). Following surgical anesthesia, the rats were placed in a rodent stereotaxic device. The skull was exposed and a small hole was made (+0.2 mm AP, −1.4 LAT of bregma). A cannula (22 g, Plastics One, Roanoke, VA) was implanted (4.2 mm deep) and anchored in place with dental cement, after which, the wound was closed with autoclips (Roboz Instruments). Following a 7 to 10 day recovery period, a second surgical procedure resulted in the insertion of a right carotid arterial catheter. The procedure used was similar to that described in mice [7] for the measurement of mean arterial pressure. The rats that were scheduled to receive intravenous infusions of compounds also had a right jugular vein catheter implanted during the same procedure in which the arterial catheter was implanted. These animals did not have the i.c.v. cannula implanted. Both the arterial and venous catheters were made from 14 cm lengths of Microrenathane (size 080, Braintree Scientific, Braintree, MA) material. Vascular catheters were flushed with heparinized saline (100 U/ml) immediately after surgery and immediately prior to testing. Rats were allowed 24 h to recover from the vascular surgery prior to testing. The venous and arterial catheter placements were verified visually at the end of the study, when the rats were euthanized. The lateral ventricular cannulae placements were also verified visually on sacrifice, following the injection of 10 µl of a methylene blue solution. Animals were excluded from the analysis if the lateral ventricles were not stained appropriately.

Cardiovascular measurements were conducted in a standard Plexiglas rodent cage identical to the rats’ home cage. Rats were placed in the recording chamber and attached to the pressure transducer (Spectromed model P23XL, Grass Instruments, West Warwick, RI) and were continuously monitored using a polygraph (Model 12RX, Grass Instruments, West Warwick RI) for the duration of the experiment. Baseline mean arterial pressure (MAP) was collected for 15 min prior to the first treatment. All i.c.v. injections were given in a volume of 10 µl. Both CRH and α-helical CRH9-41 were purchased from Calbiochem (San Diego, CA). Antalarmin was synthesized by one of the authors (K.C.R.)

Statistical significance was determined using repeated measures fixed effects MANOVA with Greenhouse-Geisser correction and subsequent post-hoc Duncan’s multiple range tests. The drug treatment conditions were loaded as the between groups factor and the time of measurement as the within-group (repeated measures) factor. All statistical analysis was conducted using CSS: Statistica (Complete Statistical Systems, Tulsa, OK) software. The criterion for statistical significance was set at P<0.05.

Fig. 1 shows the group mean (±S.E.) MAP readings in each of six rats in each of the four treatment conditions collected over the 45 min test session. CRH, 1.0 µg i.c.v., produced a statistically significant increase in MAP as compared with vehicle administration [main treatment effect: F(1,10)=9.36, P<0.02; treatment×time interaction: F(44,440)=3.18, P<0.000001]. This CRH-induced increase in blood pressure persisted in gradually reduced form for the duration of the recording session. Antalarmin blocked this increase in MAP in a dose-related fashion. Pretreatments of 10 mg/kg produced a moderate attenuation of the MAP increase elicited by CRH; the MAP change following administration of antalarmin, 10 mg/kg, and CRH was not statistically different from MAP change following vehicle pretreatment [main treatment effect: F(1,10)=0.64, P=0.45; treatment×time interaction: F(44,440)=0.72, P<0.91]. Antalarmin, 32 mg/kg, produced complete blockade of the effect of CRH, 1.0 µg i.c.v., and MAP under this condition also was not significantly different from vehicle [main treatment effect: F(1,10)=0.31, P=0.59; treatment×time interaction: F(44,440)=0.36, P<0.99]. A control experiment indicated no effect of antalarmin administration alone on MAP (data not shown); there was no significant change in baseline MAP over 45 min following a single antalarmin injection (32 mg/kg, i.p.) in four additional rats tested [main time effect: F(44,132)=1.35, P=0.10].
Fig. 1. Mean arterial pressure following i.c.v. CRH administration. Group means (±S.E.) of mean arterial pressure are plotted as a change from baseline for antalarmin doses over the course of 45 min. Antalarmin or vehicle was administered at time 0 and CRH or vehicle was administered at 15 min. Each point represents the mean of six rats. *P<0.05, **P<0.01 as compared with the vehicle-treated animals. Control mean arterial pressure (±S.E.) averaged over all experimental groups was 123.4±3.0 (n=24).

Fig. 2 shows the group mean (±S.E.) MAP in each of six rats in each of the four treatment conditions over the 45 min recording session. CRH 10 μg/kg i.v. produced a statistically significant decrease in MAP as compared with vehicle treatment [main treatment effect: F(1,10)=40.18, P<0.00009; treatment×time interaction: F(44,440)=22.82, P<0.000001]. This decrease in MAP persisted throughout the recording period (P<0.05 where noted in this effect cannot be absolutely ruled out by the present experimental findings. As a peptide, CRH has limited access to the central nervous system and parenterally administered CRH should have direct action only on this group over the duration of the recording session was peripheral CRH receptors. These are most likely involved in the depressor effect of i.v. CRH administration. This is indicated by the ability of systemic administration of the non-selective peptidic antagonist, α-CRH9–41, which also has limited access to the brain, to block this effect. The finding that antalarmin had no effect on this peripherally mediated hypotensive effect suggests that the hypotension was probably mediated through CRH2 receptors.

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References


