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Research report

The role of CRF_1 receptors for sympathetic nervous response to laparotomy in anesthetized rats

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Abstract

Corticotropin-releasing factor (CRF) is released in response to various types of stressors and mediates endocrine, autonomic, immune, and behavioral responses to stress through interaction with CRF_1 and CRF_2 receptors. To investigate the role of CRF_1 receptors in physiological responses to surgical stress, we analyzed the effects of two different non-peptide selective CRF_1 receptor antagonists (JTC-017 and CP-154,526) and a peptide non-selective CRF receptor antagonist (astressin) on laparotomy-induced sympathetic nervous responses in isoflurane-anesthetized rats. JTC-017, CP-154,526, and astressin similarly suppressed plasma ACTH elevation induced by laparotomy. JTC-017 and CP-154,526 significantly augmented plasma noradrenaline and adrenaline responses to laparotomy, while astressin showed no effect on these responses. Laparotomy-induced maximum increases in mean blood pressure and heart rate were augmented by JTC-017, but were not affected by astressin. The results suggested for the first time that there was a pathway to attenuate sympathetic nervous response to surgical stress through CRF_1 receptors in the central nervous system.

Theme: Neural basis of behavior
Topic: Stress

Keywords: Corticotropin-releasing factor; CRF_1 receptor; Sympathetic nervous response; Surgical stress; Rat

1. Introduction

Physical and emotional stresses induce endocrine and neuronal responses. In a clinical setting, a surgical insult induces profound sympathetic nervous and adrenocortical responses and may cause cardiac ischemia and hyperglycemia.

It is well known that corticotropin-releasing factor (CRF), a 41-amino acid polypeptide, plays a pivotal role in the endocrine response [33]. CRF, secreted from the paraventricular nucleus (PVN) in the hypothalamus into portal vessels, activates the hypothalamic–pituitary–adrenal (HPA) axis by triggering the immediate release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland [6,33]. CRF-containing neurons, however, widely distribute in the brain at the central nuclei of the amygdala (CeA), thalamus, hippocampus, bed nuclei of the stria terminalis (BNST), periaqueductal gray (PAG), and locus caeruleus (LC) [21,31], and are thought to regulate not only the HPA axis, but also the autonomic nervous system, immune system, and behavior including food intake, emotion, and reproduction [7,30].

CRF acts through two G-protein-coupled receptor subtypes designated as the CRF_1 and CRF_2 receptors which exhibit a different pattern of expression in the human and rat brain [30]. The CRF_1 receptor widely distributes in the central nervous system (CNS), i.e., the cortex, anterior pituitary, amygdala, cerebellum, hippocampus, olfactory

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bulb, hypothalamus, and LC [6,30], and is thought to mediate various functions in the CNS.

It has been reported that intracerebroventricularly administered CRF induced adrenergic responses, which were inhibited by an intracerebroventricularly administered CRF_1 receptor antagonist (CP-154,526) [23,34]. A non-peptide CRF_1 receptor antagonist, orally administered, attenuated the adrenergic response to psychological stress [14], suggesting that CRF may mediate or facilitate sympathetic nervous response to psychological stress by binding to the CRF_1 receptor. However, the role of the CRF_1 receptor in the sympathetic nervous response to physical stress has not been studied. Since the distribution pattern of CRF neurons activated by psychological stress differs from that activated by physical stress [19,25], it may be possible that the CRF_1 receptor in the brain plays a different role in psychological stress and physical stress.

The aim of this study was to elucidate the role of the CRF_1 receptor for the sympathetic nervous response to surgical stress in anesthetized rats by analyzing plasma catecholamine, mean blood pressure (MBP), and heart rate (HR) responses to laparotomy in the presence and absence of CRF receptor antagonists.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 280–320 g (Shimizu Laboratory Co. Ltd., Kyoto, Japan) were used. All rats were individually housed in plastic cages (30 × 20 × 20 cm) for 4–5 days under controlled temperature (25 °C) and a light schedule (lights on at 0600 h and off at 1800 h). Food and water were provided ad libitum. The study protocol was approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University.

2.2. CRF antagonists used in this study

In this study, two different non-peptide selective CRF_1 receptor antagonists (JTC-017 and CP-154,526) and a peptide non-selective CRF receptor antagonist (astressin) were used. JTC-017 was synthesized and kindly donated by Japan Tobacco Inc. (Osaka, Japan). The structure was 3,5-dichloro-*N*-(5-diethylsulfamoyl-3-dimethylamino-2-methoxyphenyl)-4-hydroxybenzamide and its molecular weight was 490 Da. The compound binds with high affinity to cloned human CRF_1 receptor (Ki = 154.7 nM) and cloned rat CRF_1 receptor (Ki = 43 nM), and inhibits CRF_1 receptor-mediated signal transduction in transfected cells (concentration that inhibits response by 50% (IC_{50}) = 3.6 nM). The compound binds with low affinity to cloned $CRF_{2\alpha}$ receptor (Ki > 100 μM). CP-154,526 (*N*-butyl-*N*-ethyl-(2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7 *H*-pyrrolol(2,3-d) pyrimidin-4-yl)-*N*-ethylamine) was synthesized and kindly donated by Pfizer Inc. (NY, USA). Astressin was purchased from Sigma-Aldrich (Tokyo, Japan). JTC-017 was dissolved in 10% hydroxypropyl-β-cyclodextrine solution (pH 9.7–10), CP-154,526 in 0.1 N hydrochloride, and astressin in distilled water. Appropriately diluted with normal saline, these drugs were administered.

2.3. Animal preparation and experimental design

Under isoflurane anesthesia, a silicone tube (Silascone tube, Kaneka Medix Corp., Osaka, Japan; inner diameter (ID) 0.5 mm, outer diameter (OD) 1.0 mm) was sanitarily inserted into the right jugular vein for infusion and blood sampling. The free end of the cannula was tunneled subcutaneously and externalized on the back of the rat. After the cannulation, rats received 10,000 IU of Benzylpenicillin potassium (Meiji Seika Kaisha Ltd., Tokyo, Japan) subcutaneously and were allowed to recover in individual cages and housed under the preconditions until the experiments.

All experiments were performed in the light phase (from 0800 h to 1500 h) 2 days after the intravenous cannulation. After induction of anesthesia in a closed box with isoflurane, the trachea was intubated with a 16-gauge intravenous cannula (Insyte™, Becton Dickinson Infusion Therapy Inc., Utah, USA) and the lungs were mechanically ventilated with a mixed gas of oxygen, air, and isoflurane (FiO_2 = 0.45) using an animal ventilator (model SN-480-7, Shinano, Tokyo, Japan). End tidal CO_2 and isoflurane concentrations were measured continuously using an anesthetic gas monitor (Type 1304, Brüel & Kjær, Naerum, Denmark) and maintained from 28 to 32 mm Hg and 1.35% (almost 1 MAC), respectively. Rectal temperature was monitored and maintained at 38 ± 0.1 °C by means of a total temperature management system. After a 120-min stabilization period, the intravenous administration of CRF receptor antagonists was started. Three different doses of JTC-017 were used. They were a 10 mg/kg bolus injection followed by an infusion at a rate of 0.6 mg/kg/min, 5 mg/kg followed by 0.3 mg/kg/min, or 2.5 mg/kg followed by 0.15 mg/kg/min. CP-154,526 was administered as a 3 mg/kg bolus injection followed by an infusion at a rate of 0.2 mg/kg/min. Astressin was administered at 100 μg/kg bolus injection alone. The injection volume was 1 ml/kg for all three drugs, and the infusion volume is described later. Thirty minutes after the drug administration, laparotomy was started. Laparotomy was performed by the procedure described in detail previously [1] with slight modifications. Briefly, the abdominal wall was opened by a vertical incision from the xyphoid process to the pubic bone (approximately 7 cm). The intestine was gently handled over its entire length while being raised from the abdominal cavity. The intestine was then replaced in the abdominal cavity. The abdomen was closed with surgical clips, and the whole process was completed within 5 min. Thirty-five minutes after the start of the laparotomy, all protocol was