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Stress induced ACTH release in capsaicin treated rats

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1 The plasma concentrations of adrenocorticotropic hormone (ACTH) in rats pretreated with capsaicin as neonates were compared with those of control rats pretreated with the capsaicin vehicle. Capsaicin pretreatment has been shown earlier to abolish the increase in plasma ACTH concentration induced by cold stress while not affecting that induced by restraint stress.

2 In the present experiments rats pretreated with capsaicin showed the same increase in plasma ACTH concentration in response to an i.v. infusion of ovine-corticotropin releasing factor as control rats pretreated with the capsaicin vehicle.

3 Intraperitoneal injection of formalin, surgical stress and intravenous infusion of (−)-isoprenaline increased plasma ACTH concentrations in control rats. In capsaicin pretreated rats the increase in plasma ACTH was significantly attenuated.

4 It is concluded that capsaicin-sensitive sensory neurones mediate the activation of pituitary ACTH secretion in response to somatosensory stimuli. The function of the corticotroph cells of the anterior pituitary is not impaired by capsaicin treatment.

Introduction

Treatment of neonatal rats with capsaicin causes irreversible destruction of peptidergic afferent C-fibres (for review see Nagy, 1982). This results in a lifelong extinction or reduction of autonomic and neuroendocrine reflex responses which are mediated by peptidergic afferent C-fibres (for review see Lembeck, 1985).

Previously, we showed that treatment of neonatal rats with capsaicin abolished the rise of plasma ACTH concentration induced by cold exposure but not that induced by restraint stress (Lembeck & Amann, 1986); basal concentrations of ACTH were not affected by pretreatment with capsaicin.

The aim of the present investigation was: (1) to determine whether capsaicin pretreatment affects the ability of the anterior pituitary gland to respond to ovine-corticotropin releasing factor (o-CRF) with an increased secretion of ACTH. Intravenous administration of o-CRF has previously been shown to stimulate ACTH release in rats with the same potency as rat-CRF (Rivier & Vale, 1985). (2) To investigate the influence of capsaicin pretreatment on ACTH secretion induced by other somatosensory forms of stress such as an i.p. injection of formalin (Berkenbosch *et al.*, 1984) or surgery. An infusion of (−)-isoprenaline (Tilders *et al.*, 1982) was used to evaluate the effect of β-adrenoceptor stimulation on ACTH release in rats pretreated with capsaicin.

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Methods

Animals

Two days old male Sprague-Dawley rats under ether anaesthesia were injected subcutaneously with either capsaicin, 50 mg kg^{-1} body weight, dissolved in a 0.15 M solution of NaCl (saline) which contained ethanol (10%) and Tween 80 (10%) or an appropriate volume (0.1 ml 10 g^{-1} body wt.) of the capsaicin solvent (vehicle) alone (Jancsó *et al.*, 1977). The rats were kept in a temperature (22°C) and light controlled (12 h light, 12 h dark) environment and had free access to a standard laboratory diet and water. At the age of three months (250–300 g), two weeks before the acute experiment, they were placed singly in individual cages. On the last 3 days before the experiment they were taken to the laboratory between 08 h 00 min and 10 h 00 min and handled by the experimenter for several minutes. The following acute experiments were all performed between 08 h 00 min and 10 h 00 min.

Types of stress

Formalin The rats were anaesthetized with sodium pentobarbitone (40 mg kg^{-1} i.p). After 15 min, 1 ml of 0.5% formalin in saline was injected i.p.; for controls, anaesthetized rats were injected i.p. with 1 ml saline. Four minutes later the rats were decapitated and blood was collected from the trunk.

Surgical stress Rats were anaesthetized as above, a midline incision was made in the neck and the left carotid artery was cannulated; 20 min later an arterial blood sample (0.6 ml) was collected.

Isoprenaline Rats were anaesthetized as above and the lateral tail vein cannulated. Saline, containing ascorbic acid (0.1 mM), was infused intravenously for 15 min followed by an infusion of (−)-isoprenaline (300 ng kg^{-1} min^{-1}, dissolved in saline containing 0.1 mM ascorbic acid) for 20 min. In control experiments the vehicle solution was infused for another 20 min instead of (−)-isoprenaline. At the end of the infusion the rats were decapitated and blood collected from the trunk. In another set of experiments only the blood pressure in the carotid artery was measured during the infusion of (−)-isoprenaline using a Statham P 23 Db pressure transducer.

Ovine-corticotrophin releasing factor Male rats (280–320 g) were anaesthetized with a combination of diazepam (6 mg kg^{-1} i.p.), morphine (20 mg kg^{-1} s.c.) and sodium pentobarbitone (25 mg kg^{-1} i.p.) in order to block the effect of unspecific stress of ACTH release (Rivier & Vale, 1985). The right jugular vein was cannulated. After an interval of 30 min o-CRF (0.1 μg or 1.0 μg in 0.1 ml of saline containing 10 mg ml^{-1} bovine serum albumin and 1 mg ml^{-1} ascorbic acid) or 0.1 ml of the o-CRF-vehicle alone was injected intravenously. Five minutes later the rats were decapitated and blood was collected from the trunk.

ACTH determination

Blood was collected in EDTA-coated tubes kept on ice. The plasma was separated by centrifugation and ACTH was measured by radioimmunoassay.

Materials

ACTH-IPR kit, Compagnie Oris Industries SA, Gif sur Yvette, France; capsaicin, Sigma Chemical Company, St. Louis, U.S.A.; diazepam, Hoffmann-LaRoche, Basel, Switzerland, morphine hydrochloride, Diosynth, Apeldoorn, Holland; o-CRF, Peninsula Inc., Belmont, U.S.A.; sodium pentobarbitone, Sanofi Sante Animale, Paris, France. (−)-Isoprenaline was a gift from Boehringer Ingelheim, Ingelheim, F.R.G. All doses of drugs refer to the base.

Statistical analysis

Two-way analysis of variance was used for statistical analysis.

Results

Ovine-corticotrophin releasing factor

The i.v. injection of o-CRF in rats anaesthetized with pentobarbitone, diazepam and morphine caused a significant increase in plasma ACTH concentrations in vehicle pretreated control rats. In capsaicin pretreated rats, the increase in plasma ACTH was of the same magnitude as in the control rats (see Figure 1).

Formalin

An i.p. injection of formalin in pentobarbitone anaesthetized, vehicle pretreated control rats caused a 4 fold increase in the plasma ACTH concentrations within

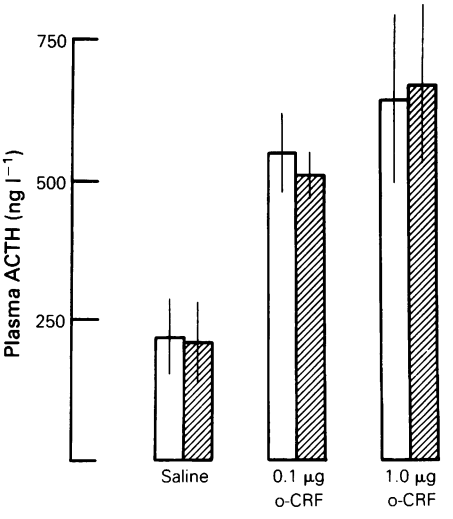

Figure 1 Plasma concentrations of ACTH in male rats (280–320 g) anaesthetized with a combination of diazepam, pentobarbitone and morphine (see Methods). Trunk blood was collected 5 min after an i.v. injection of saline, (ovine-corticotrophin releasing factor (o-CRF) solvent, see Methods), 0.1 μg or 1.0 μg o-CRF. Rats were pretreated as neonates either with capsaicin (hatched columns) or the capsaicin vehicle (open columns). Each column represents the mean, with vertical lines indicating s.e.mean ($n = 6$).

4 min. In capsaicin pretreated rats the increase was only about 2 fold (see Figure 2a).

Surgical stress

Plasma ACTH concentrations, 20 min after surgical stress (see Methods), are shown in Figure 2a. Whereas the ACTH concentration in vehicle pretreated control rats reached a mean value of 846 ng l^{-1}, in the rats pretreated with capsaicin the mean plasma concentration was only 118 ng l^{-1}, a value well within the normal range.

Effect of (−)-isoprenaline on blood pressure

An infusion of (−)-isoprenaline (300 ng kg^{-1} min^{-1} for 20 min) into the tail vein of pentobarbitone anaesthetized, vehicle pretreated control rats decreased the blood pressure in the carotid artery by 20 ± 4 mmHg ($n = 3$) and in rats pretreated with capsaicin by 18 ± 3 mmHg ($n = 3$). This indicated that capsaicin did not interfere with the cardiovascular effects of the β-adrenoceptor agonist.

Effect of (−)-isoprenaline on ACTH release

After an i.v. infusion of a total of 6 μg kg^{-1} body weight of (−)-isoprenaline for 20 min (see Methods) the plasma ACTH concentrations in vehicle pretreated control rats increased by about 3 fold. In capsaicin pretreated rats the increase was significantly smaller; in fact, the mean plasma ACTH concentration was not significantly higher than that of capsaicin pretreated rats which were infused with the (−)-isoprenaline solvent alone (see Figure 2b).

Discussion

Stressful stimuli cause an increase in plasma ACTH concentration. The stimuli can be either of a somatosensory nature, e.g. exposure to a cold environment or pain, or they can be predominantly emotional, e.g. mild restraint stress (Berkenbosch *et al.*, 1984). Both forms of stress activate various hypothalamic neurones which control the release of several releasing factors into the hypophyseal portal vein, and finally the secretion of ACTH from the anterior pituitary gland. It can be assumed that the neuroendocrine response to 'emotional' stress involves mainly neurones within the central nervous system, whereas somatosensory forms of stress involve, in addition, sensory pathways between the periphery and the brain (for review see Feldman & Saphier, 1985).

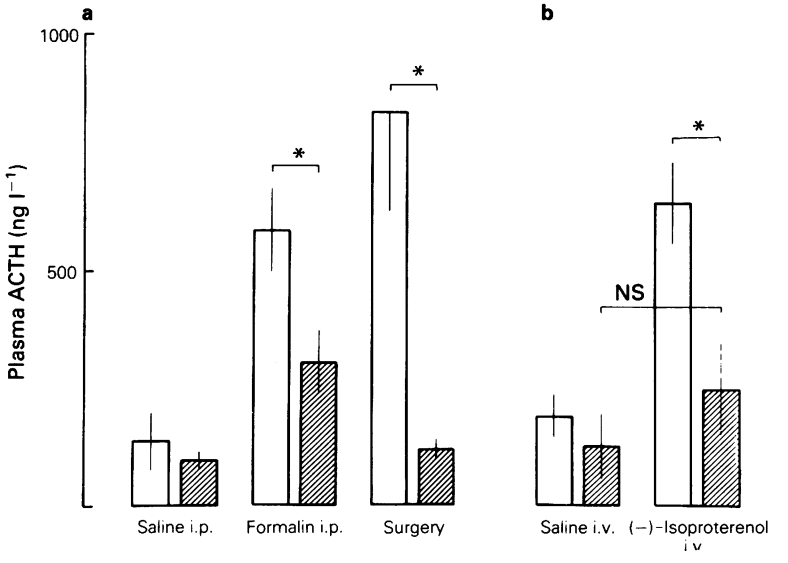

Figure 2 Plasma concentrations of ACTH in male rats (250–300 g) treated as neonates either with capsaicin (hatched columns) or the vehicle (open columns). All rats were anaesthetized with sodium pentobarbitone. (a) Effect of saline i.p., formalin i.p. and surgery. (b) Effect of i.v. infusion of saline ((−)-isoprenaline solvent) and (−)-isoprenaline; (for details see Methods). Each column represents the mean with vertical lines indicating s.e.mean; $n = 6$ in each group. Significance of difference between capsaicin pretreated and vehicle-pretreated group: $^*P < 0.05$ (two-way analysis of variance). The difference between the capsaicin treated rats injected with either saline or (−)-isoproterenol in (b) was not significant (NS).

Previous experiments have demonstrated that in rats, pretreated as neonates with capsaicin, the release of ACTH normally evoked by a mild restraint stress was unimpaired, whereas ACTH release in response to cold was significantly reduced (Lembeck & Amann, 1986).

The present experiments have shown that in anaesthetized rats the ACTH response to other 'somatosensory' stress forms, such as i.p. injection of formalin or surgery, was also diminished if the animals had been pretreated with capsaicin. Furthermore, capsaicin pretreatment did not interfere with the release of ACTH from the corticotroph cells of the anterior pituitary gland in response to o-CRF (see Figure 1).

Capsaicin treatment of neonate rats causes a lifelong depletion of neuropeptides, such as substance P and somatostatin, in sensory neurones, but not in the hypothalamus (Gamse *et al.*, 1981). Thus it appears that the action of capsaicin on afferent sensory neurones may be the reason for the attenuation of the ACTH response to 'somatosensory' stress forms in capsaicin pretreated rats.

In addition, the present experiments have shown that ACTH release, induced by stimulation of β-adrenoceptors with isoprenaline is considerably diminished in capsaicin pretreated rats. The mechanisms by which β-adrenoceptor stimulating drugs cause ACTH release are still disputed. A direct action on the anterior pituitary gland has been suggested (Mezey *et al.*, 1983; Axelrod & Reisine, 1984) as well as an indirect action (Tilders *et al.*, 1985) possibly involving peripheral receptors (Tuomisto & Mannistö, 1985). The hypotension caused by β-adrenoceptor stimulation was suggested as a possible cause for ACTH release by Knepel *et al.* (1982).

In the present experiments the decrease in blood pressure after isoprenaline infusion was similar in control and in capsaicin pretreated rats. If the fall in blood pressure were the trigger for ACTH release, then it could be assumed that this event was conveyed to the brain via capsaicin sensitive afferent neurones which had been affected by pretreatment.

The involvement in neuroendocrine mechanisms of afferent, capsaicin-sensitive neurones has been demonstrated on previous occasions. Thus, the copulation-induced release of prolactin, which involves sensory neurones (Vogt, 1933), was found to be absent in capsaicin pretreated rats (Traurig *et al.*, 1984a,b; 1986). Furthermore, the increase in adrenaline secretion during insulin-induced hypoglycaemia was shown to be diminished in capsaicin pretreated rats. It has been proposed that this was due to the impairment of capsaicin-sensitive afferent neurones originating at peripheral glucose receptors (Amann & Lembeck, 1986).

Our present studies on stress, together with previous observations (Lembeck & Amann, 1986) suggest that the ACTH release following 'somatosensory' forms of stress may also be dependent on the normal function of capsaicin-sensitive afferent neurones.

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