The aim of the present study was to compare the effect of social stress on the corticotropin releasing hormone (CRH) and arginine vasopressin (AVP)-induced pituitary-adrenocortical activity. Also the significance of prostaglandins (PG) generated by constitutive and inducible cyclooxygenase (COX-1 and COX-2) in the stimulation of hypothalamic-pituitary-adrenal (HPA) axis by AVP under basal and crowding stress conditions was investigated. The control rats were housed 7 in a standard cage and stressed rats were crowded 24 in a cage of the same size during 7 days. The activity of HPA axis was determined by measuring plasma ACTH and serum corticosterone levels 1 h after i.p. AVP administration. Indomethacin (2.0 mg/kg i.p.), a non-selective COX inhibitor, piroxicam (0.2, 2.0, and 5.0 mg/kg), a more potent COX-1 than COX-2 inhibitor, and compound NS-398 (0.2 and 2.0 mg/kg) a selective COX-2 inhibitor, were administered i.p. 15 min prior to AVP (5.0 µg/kg i.p.) to control or crowded rats. The obtained results indicate that social stress for 7 days considerably inhibits the stimulatory action of AVP on ACTH secretion, while it intensifies the CRH-induced ACTH secretion. Indomethacin, piroxicam and NS-398 significantly diminished the AVP-elicited ACTH and corticosterone secretion in non-stressed rats. None of these COX antagonist induced any significant inhibition of the AVP-induced ACTH and corticosterone secretion in stressed rats. Therefore, PG generated by COX-1 or COX-2 do not participate to a significant extent in the HPA stimulation by AVP during crowding stress. These results suggest that social crowding stress desensitizes the PG stimulatory mechanism which considerably mediates the AVP-induced HPA stimulation under basal conditions. The results contrast with a lack of any involvement of PG in the CRH-induced stimulation of HPA response under basal or crowding stress conditions.

Key words: social stress, prostaglandins, COX-1, COX-2, corticotropin releasing hormone, vasopressin, ACTH, corticosterone
INTRODUCTION

The main factor controlling ACTH release from the anterior pituitary under acute stress is CRH synthesized in the hypothalamic parvocellular paraventricular nucleus (pPVN). Vasopressin (AVP) is co-localized with CRH in approximately half of CRH positive parvocellular cells of normal resting animals (1). Single acute immobilization stress is sufficient to up-regulate AVP as well as CRH mRNA production in the hypothalamic CRH-neurosecretory system. However, during stress the number of AVP-expressing parvocellular CRH neurons increases more potently than AVP-deficient CRH neurons (2). Single stressful stimuli also increase the AVP stores in terminals of CRF neurons in the external zone of the median eminence (3). Vasopressin acts synergistically with CRH at the anterior pituitary corticotrophs to release ACTH. It has been proposed that AVP-containing subset of CRH neurons are selectively activated in response to stress, and growing evidence indicate increased role for AVP during chronic stress (4). Vasopressin is proposed to be important for maintaining the activity of the HPA axis after repeated stimulation. (5). During repeated or chronic stress, there is a shift from non-AVP-producing CRH neurons, and increase in AVP vesicles in the median eminence (6) and a release of AVP, but not of CRF (7).

We have found that in rats crowded for 3 days the CRH-induced ACTH response was moderately increased and corticosterone response remained unaffected, indicating that CRH system remains fully sensitive or hyperactive. By contrast, crowding stress considerably reduced the AVP-induced ACTH and corticosterone response (8, 9).

Vasopressin stimulates pituitary ACTH secretion after binding to V\textsubscript{1b} vasopressin receptors coupled to phospholipase C which activates inositol phosphate formation.

Regulation of pituitary vasopressin V\textsubscript{1b} receptors plays a crucial role in regulating pituitary adrenocorticotropic hormone (ACTH) secretion during adaptation to stress (10, 11).

Prostaglandins are released under stressful circumstances and they mediate ACTH and corticosterone response to psychological stress (13) and physiological response to exercise (14). We have recently found that prostaglandins generated by constitutive or inducible cyclooxygenase are involved in the adrenergic and cholinergic stimulation of HPA axis in non-stressed and crowded rats (15-17). However, PG do not mediate the stimulation of HPA axis by CRH under basal or social stress conditions. Although indomethacin, a non-selective COX inhibitor, significantly impairs the AVP-induced ACTH and corticosterone secretion, the involvement of PG generated by COX-1 and COX-2 in the stimulation of HPA axis by AVP under basal conditions and during social crowding stress is not known.

In this study we compared the effect of crowding stress on the AVP- and CRH-induced ACTH and corticosterone response. We also investigated the involvement of PG generated by COX-1 and COX-2 in the AVP-induced ACTH and corticosterone response under basal conditions and during social crowding stress.
MATERIALS AND METHODS

Male Wistar rats with initial body mass of 180-210 g were housed 7 per cage and maintained under controlled conditions, with light period from 7.00 to 19.00 h at a temperature of 20±2°C. The animals were provided with unlimited access to commercial food and tap water. The rats were given a one-week acclimation period before the onset of experimentation. Animal care and handling throughout the experimental procedures were in accordance with bioethical requirements. The experimental protocols were approved by the local Ethics Committee.

Treatment

The rats were randomly assigned to control or crowding stress group. The control rats were housed 7 per cage (52x32x20 cm) and remained in their home cages until scheduled for treatment. The stressed rats were crowded in groups of 24 per cage of the same size for 7 days, since after that time we found a potent impairment of the HPA responsiveness to neuropeptide and neurotransmitter receptors stimulation. The effect of cyclooxygenase blockers on the vasopressin (AVP)-induced ACTH and corticosterone response in crowded rats were compared with the effects in control non-stressed animals. For this purpose 15 min prior to AVP (5 µg/kg i.p.) both non-stressed and crowded rats were pretreated with indomethacin (2 mg/kg i.p.), a non-selective cyclooxygenase inhibitor or piroxicam, a preferential constitutive cyclooxygenase (COX-1) blocker (0.2, 2.0 and 5.0 mg/kg i.p.), and compound NS-398, a selective inducible cyclooxygenase (COX-2) blocker (0.2 and 2.0 mg/kg i.p.).

ACTH and corticosterone determinations

One hour after the last injection the rats were decapitated immediately after their removal from the cage and their trunk blood samples were collected on ice in plastic conical tubes containing 200 µg of a solution of 5 mg/ml EDTA and 500 TIU of aprotinin (Sigma). Control rats were decapitated concurrently with the experimental group. Plasma was separated by centrifugation in a refrigerated centrifuge within 30 min and frozen at -80°C until the time of assay. Plasma ACTH concentrations were measured using the double antibody $^{125}$I radioimmunoassay obtained from CIS Bio International and calculated as pg/ml of plasma. The concentration of serum corticosterone was measured fluorometrically and expressed as µg per 100 ml. To avoid circadian variability, all experiments were performed between 10-11 a.m. and all decapitations between 11-12 a.m., when plasma hormones are at a relatively low levels.

Preparation of drugs

Drugs used in this study: corticotropin-releasing hormone, human, rat sequence (rCRH), arginine vasopressin (AVP), indomethacin, piroxicam and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (Steinheim, Germany) and compound NS-398 was purchased from Cayman Chemical Co (Ann Arbor Mi, USA). Piroxicam and indomethacin were prepared for injection by sonication in 1% Tween solution, NS-398 was dissolved in DMSO and CRH and AVP were dissolved in saline. The doses used are expressed in terms of salts. Solutions were prepared immediately before use. The required doses of drugs or solvents were injected i.p. in a volume of 2 ml/kg.

Statistics

The results were calculated as a group mean ± standard error of the mean. Statistical evaluation was performed by an analysis of variance, followed by individual comparision with Duncan’s test. P values less than 0.05 were taken to indicate statistical significance.
RESULTS

Effect of crowding stress on the AVP- and CRH-induced ACTH and corticosterone response

In rats crowded for 7 days AVP (5 µg/kg) given i.p. evoked significantly lower ACTH and corticosterone secretion, by 30 and 31.3%, respectively, compared with the AVP-induced responses in control non-stressed rats (Fig. 1). By contrast, crowding stress markedly increased the CRH (1 µg/kg i.p.)-induced ACTH secretion (by 18%) and slightly diminished the corticosterone response (by 8.1%) compared with the CRH-elicited response in control non-stressed rats. (Fig. 1).

Effect of indomethacin on the AVP-induced ACTH and corticosterone response

Pretreatment with indomethacin (2.0 mg/kg i.p.), a non-selective cyclooxygenase antagonist, considerably decreased the AVP-induced ACTH and corticosterone

Fig. 1. The AVP- and CRH-induced ACTH and corticosterone response in rats crowded for 7 days. AVP and CRH were injected i.p. 1 h before decapitation. Values represent the mean ± SEM of 6 rats. ++p<0.01 vs. saline treated groups; ^^p<0.01 vs. AVP-induced responses in control, non-stressed rats.
secretion. In non-stressed rats indomethacin reduced ACTH secretion by 59% and corticosterone secretion by 52%. In stressed rats indomethacin slightly increased the AVP-induced ACTH response (+7.9%) and decreased (-12.4%) corticosterone response, compared with the reduction in non-stressed rats (Fig. 2).

The piroxicam-induced ACTH and corticosterone response to AVP in control and stressed rats.

Piroxicam (0.2-5.0 mg/kg i.p.) which is stronger COX-1 antagonist than indomethacin, also gradually decreased the AVP-induced ACTH and corticosterone response by up to 41.3% and 42.7% in control, non-stressed rats. In rats crowded for 7 days piroxicam (5 mg/kg i.p.) markedly, though not significantly, diminished the AVP-induced ACTH response (by 27.8%) but did not substantially alter the AVP-elicited increase in corticosterone response (Fig. 3). This finding suggests that crowding stress significantly diminished the involvement of endogenous PG generated by COX-1 in the AVP-induced HPA response.

![Fig. 2. Effect of indomethacin on the AVP-induced ACTH and corticosterone secretion in rats crowded for 7 days. Indomethacin was injected i.p. 15 min before AVP and 1 h later the rats were decapitated. ++p<0.01 vs. solvent treated group; **p<0.01 vs. AVP treated group; ^p<0.05 vs. AVP-treated non-stressed group.](image-url)
Effect of compound NS-398 on the AVP-induced ACTH and corticosterone secretion

Pre-treatment of rats with a selective COX-2 blocker, compound NS-398 (0.2 and 2.0 mg/kg i.p.) moderately decreased the AVP-induced ACTH and corticosterone response in non-stressed rats, by up to 22.2% and 19.1%, respectively. In rats crowded for 7 days this blocker did not markedly alter the AVP-induced ACTH or corticosterone response; it diminished ACTH secretion by 3.2% and moderately increased corticosterone secretion, by 22.0% (Fig. 4).

DISCUSSION

The present study demonstrated that social crowding stress significantly impaired the HPA axis response to vasopressin but not CRH. In rats exposed to
crowding stress for 7 days the AVP-induced ACTH and corticosterone secretion was diminished by 30 and 31.3%, respectively, compared with the secretion in non-stressed controls. A similar diminution of the AVP-induced HPA response was also observed in rats after 3 days of crowding stress in our former experiment (8, 9). On the other hand crowding stress for 7 days markedly increased the CRH-induced ACTH secretion and did not significantly alter corticosterone secretion (18). Likewise, crowding stress of shorter duration (3 days) did not diminish the CRH-induced HPA axis response (8). Chronic social stress altered the levels of CRH and AVP mRNA in rat brain (19). Also novel environment stress affects AVP mRNA in the hypothalamic paraventricular nucleus, indicating the involvement of central mechanism of AVP activity during social stress (20).

Our present results suggest that hypersecretion of AVP activates the stress response of HPA axis during chronic stress and results in a significant desensitization of the AVP-elicited HPA axis response. It is not known to what
extent chronic crowding stress increases the secretion of AVP which induces AVP receptors desensitization. Alterations of the number of vasopressin V$_{1b}$ receptors in the pituitary especially during chronic stress, may affect pituitary corticotroph responsiveness. Prolonged crowding stress may initially upregulate vasopressin receptor and increase V$_{1b}$ receptor mRNA followed by a fall in V$_{1b}$ receptor number and sensitivity (11). Although the molecular mechanisms responsible for V$_{1b}$ receptor regulation are unknown a high correlation exists between the number of V$_{1b}$ receptors and ACTH responsiveness to stress.

The activation of HPA axis manifested by elevated plasma ACTH and corticosterone levels in the present experiment was observed on the onset of crowding but not in the following days. It is not clear whether the initial rise in corticosterone levels during crowding stress was able to inhibit, by a fast negative feedback, the stimulatory action of AVP. It is possible, but not proved, that a short lasting corticosterone hypersecretion is sufficient to reduce the response of HPA axis to exogenous AVP like it abolished the response to adrenergic and cholinergic agonists (21).

Indomethacin, a non-selective COX inhibitor, considerably diminished the AVP-induced ACTH and corticosterone secretion in non-stressed rats. In rats crowded for 7 days the pituitary-adrenocortical response to AVP was significantly diminished and indomethacin did not markedly alter ACTH (+7.9 %) and corticosterone response (-12.4%). This observation suggests that endogenous PG significantly mediate the AVP-induced pituitary-adrenal hormones secretion in control but not in stressed rats. Therefore, the impairment of the AVP-induced HPA response during crowding stress may result from desensitization of PG systems involved in the AVP-induced pituitary-adrenocortical axis activation under basal conditions.

Piroxicam, which acts more potently as COX-1 than COX-2 blocker in different cells, also significantly diminished the AVP-induced ACTH and corticosterone response in non-stressed rats. This diminution was somewhat lesser than that induced by indomethacin, suggesting that indomethacin may also act via inhibiting the generation of PG by COX-2 isoenzyme. In fact compound NS-398 markedly diminished the AVP-induced ACTH and corticosterone secretion in non-stressed rats. In crowded rats piroxicam evoked slightly lesser inhibition of the AVP-induced ACTH secretion than in control rats and did not substantially alter corticosterone secretion. Exogenous AVP may stimulate catecholamine secretion from the adrenal gland medulla where functional V$_{1b}$ AVP receptors found in chromaffin cells, may also be involved in ACTH secretion. Moreover AVP may stimulate corticosterone secretion in rat zona glomerulosa by acting on the V$_{1a}$ receptor subtype (22). It is not known yet whether, or to what extent, chronic social stress affects this autocrine-paracrine action of AVP but this may represent a mechanism independent from the hypothalamic-pituitary part in the local regulation of corticosterone secretion. This autocrine-paracrine mechanisms
may result in the dissociation of ACTH-corticosterone alterations in response to AVP and COX-inhibitors in stressed rats observed in the present experiment.

The obtained results indicate that social crowding stress considerably impairs the stimulatory action of AVP on ACTH secretion while it increases the CRH-induced ACTH secretion. These results suggest that social crowding stress abolishes the mediation by endogenous PG of the AVP-induced HPA stimulation observed under basal conditions.

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