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PROSTAGLANDINS AND INTERLEUKIN-1β IN THE HYPOTHALAMIC-PITUITARY-ADRENAL RESPONSE TO SYSTEMIC PHENYLEPHRINE UNDER BASAL AND STRESS CONDITIONS

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We investigated the role of interleukin-1β (IL-1β) and prostaglandins (PG) in the α1-adrenergic agonist, phenylephrine-induced hypothalamic-pituitary-adrenal axis (HPA) responses under basal and social stress conditions. Male Wistar rats, either control or exposed to crowding stress for 7 days prior to treatment, were used in these experiments. All compounds were injected i.p. Cyclooxygenase COX-1 and COX-2 inhibitors, piroxicam and compound NS-398, IL-1β and IL-1β receptor antagonist (IL-1βRA) were injected 15 min before phenylephrine. Plasma ACTH and serum corticosterone levels were measured 1 h after phenylephrine or IL-1β injection.

Phenylephrine, in respective higher dose administered systemically (0.4 mg/kg i.p.) was almost equally effective as given i.c.v. (30 µg) in stimulating ACTH and corticosterone secretion. Likewise, the extent of the involvement of PG generated by COX-1 and COX-2 in the phenylephrine-induced ACTH and corticosterone secretion was similar after systemic or i.c.v. treatment under both resting and stress conditions. Piroxicam, stronger than compound NS-398, reduced the i.p. phenylephrine-induced ACTH and corticosterone secretion. IL-1β receptor antagonist (50 µg/kg i.p.) did not significantly affect the inhibitory action of piroxicam on the i.p. phenylephrine-induced ACTH and corticosterone secretion in control rats, but significantly enhanced the inhibition evoked by piroxicam in stressed rats. IL-1β (2.5 µg/kg i.p.) significantly increased ACTH and corticosterone secretion under basal conditions. Crowding stress for 7 days markedly impaired the IL-1β-induced ACTH and corticosterone secretion. The mechanism of the stimulatory action of i.p. IL-1β, which does not cross the blood-brain barrier, may comprise both central and peripheral components of the HPA axis. These results suggest that under basal conditions IL-1β is not markedly involved in the α1-adrenergic agonist-induced stimulation of the HPA axis activity. During social crowding stress IL-1β and prostaglandins are significantly involved in this stimulation.

Key words: α1-adrenergic agonist, COX-1, COX-2 inhibitors, interleukin-1β receptor antagonist, IL-1β and PG in HPA stimulation
INTRODUCTION

Prostaglandins generated by constitutive and inducible cyclooxygenase (COX-1 and COX-2) are significantly involved in central stimulation of the hypothalamic-pituitary-adrenal (HPA) axis by adrenergic systems (1, 2). Under basal conditions the stimulation of ACTH and corticosterone secretion by phenylephrine, an $\alpha_1$-adrenergic agonist, given intracerebroventricularly was considerably reduced by COX-1 and COX-2 inhibitors, piroxicam and compound NS-398 (3). Social crowding stress did not alter significantly the involvement of prostaglandins in the HPA response to stimulation of central $\alpha_1$-adrenergic receptors (4).

Interleukin (IL)-1, a polypeptide predominantly produced by activated monocytes and macrophages activates the HPA axis. Actually, the IL-1 family consists of three different genes located in human on the long arm chromosome 2, which encode three distinct proteins with structural homologies. IL-1$\alpha$ and IL-1$\beta$ act as agonist molecules. The third member of the family, IL-1 receptor antagonist (IL-1RA) that competitively antagonizes binding of IL-1$\alpha$ and IL-1$\beta$ to the IL-1 receptor (5). It is devoid of any biological activity and therefore acts as an endogenous inhibitor of the IL-1 activity (6, 7). The IL-1 receptor antagonist (IL-1RA) has the same molecular weight (17.5 kD mol. wt) and three-dimensional structure as IL-1$\beta$.

Due to its size and structure, peripherally produced or applied IL-1$\beta$ cannot cross the blood-brain barrier (BBB) passively (8 -10). Several mechanisms have been proposed for IL-1 stimulation of the brain and subsequent CNS effects: penetrance of IL-1 at one or more circumventricular organs which do not have a normal BBB or activation of IL-1 receptors on endothelial cells of brain blood vessels and transduction of a signal which stimulates brain pathways. Some data suggest that rhIL-1$\beta$ activates the HPA axis via stimulation of peripheral vagal afferents and further support the hypothesis that peripheral cytokine signaling to the CNS is mediated primarily by stimulation of peripheral afferents (11, 12). Also stimulation of peripheral, cholinergic pathways may be transmitted by the vagus afferents to stimulate central limb of the HPA axis (13). Centrally applied IL-1$\beta$ can enter the brain parenchyma and act as a volume transmission signal to activate the HPA axis (14).

Interleukin-1$\beta$ plays an important role in hypothalamic neuronal regulation of the stress response primarily, but not exclusively, by increasing secretion of corticotrophin-releasing hormone (CRH) from the median eminence as a result of actions in the paraventricular nucleus of the hypothalamus (PVN) (15, 16).

Central pathways involved in the IL-1$\beta$-induced activation of the HPA axis are, at least in part, dependent upon prostaglandin systems (17 - 20). When injected into the brain, the PGE$_2$ triggered transcription of CRH and its type 1 receptor essentially in the hypothalamic PVN. Local production of PGE$_2$, is therefore likely to be a crucial step within the CNS to mediate the effects of
circulating cytokines on the neuronal circuitry mediating the activation of HPA (17). The limited entry of interleukin-1β into the central nervous system has led to the hypothesis that peripheral IL-1β acts, through IL-1β receptors located on endothelial cells, where it activates the release of prostaglandins which in turn stimulate the HPA axis (19). IL-1 receptor and COX-2 enzyme are colocalised in the PVN.

Our former study showed a significant involvement of central PG in the α1-adrenergic stimulation of HPA axis by i.c.v. phenylephrine (3). The mediation of prostaglandins in the hypothalamic-pituitary-adrenal axis by interleukin-1β activation of cyclooxygenase and PG production is generally accepted. In the present study we investigated a functional role of IL-1β and PG generated by COX-1 and COX-2 in the α1-adrenergic stimulation of HPA axis under basal and social stress conditions. A further purpose of the present experiments was to compare the functional involvement of prostaglandins generated by COX-1 and COX-2 isoenzymes in the systemic and central α1-adrenergic stimulation of ACTH and corticosterone secretion under basal and stress conditions.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 190-220 g at the beginning of experiment were used in these studies. The animals were housed in standard laboratory conditions 7 per cage and were provided with unlimited access to commercial food and tap water. The animal room was maintained on a 12-hour light dark cycle beginning at 7 a.m. and temperature of 20 ± 2°C. All animals were given a one-week acclimation period before the onset of experimentation. The experiments procedures were performed in accordance with bioethical requirements and were approved by the Institutional Ethics Committee.

Experimental design

The rats were randomly assigned to one of two experimental groups: control and social stress of crowding. Control rats were housed 7 per cage (52x32x20 cm) and remained in their home cages until scheduled for treatment. 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 some monoaminergic and cholinergic stimulation.

Experiments were carried out in three separate series. First series of experiment was performed to determine the effect of constitutive (COX-1) and inducible (COX-2) cyclooxygenase on the i.p. phenylephrine-induced pituitary-adrenocortical stimulation in non-stressed and crowded rats. Control rats, intact or crowded, were injected i.p. with saline (0.9% NaCl or 1% Tween 80, 2 ml/kg). Second series of experiments was performed to determine the effects of IL-1β on ACTH and corticosterone secretion in rats under resting and stress conditions. In the third group the effect IL-1β receptor antagonist and COX-1 antagonist, piroxicam on the phenylephrine-induced ACTH and corticosterone secretion in non-stressed and crowded rats were compared. The IL-1 receptor antagonist, (IL-1βRA) which competitively blocks both IL-1β and 1α receptors from binding to IL-1R (5), was used to examine the functional involvement of IL-1β in the adrenergic-induced stimulation of ACTH and
corticosterone secretion. Piroxicam, a preferential COX-1 inhibitor, compound NS-398, a selective COX-2 inhibitor, and IL-1β receptor antagonist were injected systemically 15 min before phenylephrine. One hour after the last injection the rats were decapitated immediately after their removal from the cage and their trunk blood was collected.

**Drugs and solution**

Drugs used in this study were: piroxicam (Sigma), compound NS-398 (Cayman Chemical Co), interleukin-1β (IL-1β) monoclonal mouse recombinant and IL-1β antagonist monoclonal mouse recombinant (Sigma). The doses used are expressed in terms of salts. Piroxicam was prepared for injections by sonication in 1% Tween solution, NS-398 was dissolved in DMSO and phenylephrine was dissolved in saline. Solutions were prepared immediately before use. The drugs or solvents were administered i.p. in a volume of 2 ml/kg.

**ACTH and corticosterone determinations**

Trunk blood samples were collected on ice to plastic conical tubes containing 200 µl 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 -20°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.

**Statistics**

The effects of cyclooxygenase blockers and IL-1β receptor antagonist on the phenylephrine-induced ACTH and corticosterone responses in crowded rats were compared with the respective effects in control animals. 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 comparison with Duncan’s test. The results were considered to be significantly different when P<0.05.

**RESULTS**

**Effect of COX-1 and COX-2 inhibitors on the systemic phenylephrine stimulated ACTH and corticosterone secretion**

Piroxicam, a predominantly selective COX-1 inhibitor, given i.p. (0.2 - 2.0 mg/kg) 15 min before phenylephrine (0.4 mg/kg i.p.), an α₁-adrenergic receptor agonist, gradually decreased, by 31.7 to 50.7%, the phenylephrine-induced significant increase in plasma ACTH levels in conscious rats (Fig. 1). The piroxicam-evoked decrease in the phenylephrine-induced corticosterone secretion (8.5 - 33.3%) was also dose-dependent but less pronounced than the corresponding decrease in ACTH level (- 50.7%). This result may suggest that corticosterone secretion does not depend only on an actual plasma ACTH levels (Fig. 1).
A selective COX-2 inhibitor compound NS-398 (0.2 - 2.0 mg/kg), given i.p. 15 min earlier also significantly reduced the phenylephrine-induced ACTH secretion, nearly to the same extent, after both doses (by 45.7 and 44.4%, respectively). This COX-2 inhibitor reduced the phenylephrine-induced corticosterone secretion (by 54 and 44.1%, respectively) in a manner parallel to the impairment of ACTH secretion (Fig. 1).

**ACTH and corticosterone secretion after systemic or central administration of phenylephrine and COX inhibitors under basal and stress conditions**

In this series of experiment the involvement of PG in the stimulatory action of phenylephrine were compared when both phenylephrine and COX inhibitors were administered either systemically or intracerebroventricularly in rats under resting or stress conditions. Under basal conditions piroxicam (0.2 mg/kg) given i.p. 15 min earlier considerably decreased the i.p. phenylephrine-induced ACTH secretion (50.4%). This inhibition was stronger than that induced by *i.c.v.* piroxicam (0.2 µg) given before phenylephrine (30 µg) administered by the same route (30%) (Fig. 2). Piroxicam (0.2 mg/kg i.p.), also significantly decreased (-33.3%) the phenylephrine (0.4 mg/kg i.p.)-elicited corticosterone secretion, comparably to the respective diminution (45%) of the phenylephrine (30 µg *i.c.v.*)-induced corticosterone secretion by *i.c.v.* piroxicam (0.2 µg) (Fig. 2).

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**Fig. 1.** Effect of piroxicam (PIROX) and compound NS-398 on the phenylephrine (PHEN)-induced ACTH and corticosterone secretion. Piroxicam and NS-398 were injected i.p. 15 min before i.p. phenylephrine. In Fig. 1 - 5 one hour after the last injection the rats were decapitated. Values represent the mean ± SEM of 6 rats. ++P<0.05 vs. solvent-treated group. *P< 0.05 and **P<0.01 vs. phenylephrine-treated group.
In rats crowded for 7 days piroxicam administered i.p. (0.2 mg/kg) or i.c.v. (0.2 µg) 15 min earlier significantly decreased the ACTH response to phenylephrine given either i.p. or i.c.v. (-45% or 55%, respectively) (Fig. 2). The corresponding inhibitions by piroxicam of the phenylephrine-induced corticosterone responses paralleled the reductions in ACTH responses, but were less pronounced (-14%, and -26%, respectively).

In rats under basal conditions inhibitor of COX-2, compound NS-398 (0.2 mg/kg) given i.p. induced a weaker decrease of the i.p. phenylephrine (0.4 mg/kg)-induced ACTH response (17%) than administered (0.1 µg) i.c.v. (29.9%). Compound NS-398 (0.2 mg/kg) injected i.p. did not diminish the i.p. phenylephrine-induced corticosterone secretion (+6.5%) and given i.c.v. (0.1 µg) significantly decreased (-25.3%) this secretion (Fig. 2). In stressed rats this inhibitor did not markedly alter the i.p. or i.c.v. phenylephrine-induced ACTH (-25.3% and -21%) and corticosterone secretion (+10.9% and -13%, respectively) (Fig. 2).

**Effect of IL-1β on ACTH and corticosterone secretion in stressed rats**

Interleukin 1β (2.5 µg/kg i.p.) considerably increased ACTH and corticosterone secretion in rats under resting conditions. Crowding stress for 7
days significantly diminished the IL-1β-induced ACTH response (-19%) and corticosterone response (-33%), indicating that substantial, but not major, part of stress-induced desensitization of HPA axis to IL-1β stimulation may comprise the IL-1β and PG systems (Fig. 3).

**Effect of interleukin 1β (IL-1β) antagonist on the phenylephrine-induced ACTH and corticosterone secretion**

Interleukin 1β receptor antagonist (5-50 µg/kg) given systematically to nonstressed rats did not significantly alter the resting plasma ACTH and serum corticosterone levels (data not shown). Likewise, pretreatment with IL-1βRA (5-50 µg/kg i.p.) given 15 min earlier did not significantly affect the stimulatory effect of i.p. phenylephrine (0.4 mg/kg) on ACTH secretion in control rats. The IL-1βRA antagonist-induced changes did not exceed +16.2% to -15.9% of the phenylephrine-induced effect (Fig. 4). Similarly, IL-1βRA did not markedly alter the phenylephrine-induced corticosterone response (-24.5 to +16.1%) (Fig. 4).

**Fig. 3.** Stimulatory action of IL-1β on ACTH and corticosterone secretion in control and stressed rats. ++P<0.01 vs. solvent-treated control group; *P<0.05 and **P<0.01 vs. IL-1β-treated control group.
Effect of IL-1β receptor antagonist (A-IL-1β) on the phenylephrine-induced ACTH and corticosterone response. ++P<0.05 vs. solvent-treated group.

**Effect of IL-1βRA and piroxicam on the phenylephrine-induced HPA response in stressed rats**

Piroxicam, a COX-1 inhibitor (2.0 mg/kg) given i.p. 15 min earlier to nonstressed rats abolished the phenylephrine-induced ACTH secretion and significantly reduced corticosterone response. IL-1βRA (50 µg/kg i.p.) did not alter the phenylephrine- or phenylephrine + piroxicam ACTH response and intensified the piroxicam-induced reduction of corticosterone response (-60.8%).

In rats crowded for 7 days IL-1βRA decreased the phenylephrine-induced ACTH response by 39.8% whereas piroxicam (2 mg/kg i.p.) alone moderately diminished this response (14%). Combined pretreatment of stressed rats with IL-1βRA and piroxicam induced the most pronounced decrease in ACTH response to phenylephrine and similarly suppressed corticosterone secretion. IL-1βRA (10 µg/kg i.p.) induced a stronger decrease of the phenylephrine-induced corticosterone secretion (-66.7%) than piroxicam (-35.5%). A combined pretreatment of rats with both these antagonist almost totally suppressed the phenylephrine-induced corticosterone secretion in stressed rats (Fig. 5).

**DISCUSSION**

The present results show that phenylephrine, an α₁-adrenergic receptor agonist, given i.p. (0.4 mg/kg) to conscious rats significantly increased ACTH
and corticosterone secretion 1 h after injection. This increase was moderately weaker than that induced by i.c.v. phenylephrine (30 µg) administration (3, 4). This suggests that phenylephrine given systemically in effective doses, can activate both central and peripheral limb of the HPA axis, thought the increase in ACTH and corticosterone secretion after i.c.v. phenylephrine administration was stronger than that after systemic injection. Phenylephrine given into lateral cerebral ventricle can easily reach $\alpha_1$-adrenergic receptors in central structures involved in the activation of HPA axis. During adrenergic stimulation high levels of $\alpha_{1A}$- and $\alpha_{1B}$-adrenergic receptor mRNA were found in the hypothalamic PVN where CRH is synthesized (21, 22). The stimulatory mechanism of phenylephrine given i.p. on HPA axis has not been elucidated. This agonist administered systemically may directly stimulate ACTH release from CRH neurons in the median eminence which is devoid of the blood-brain-barrier (BBB). It is not clear to what extent phenylephrine may penetrate the BBB to stimulate ACTH secretion indirectly by activating $\alpha$-adrenergic receptors in the hypothalamic PVN. Peripheral $\alpha$-adrenergic stimulation may also be transduced via vagal afferents to central structures involved in HPA axis activation (11, 13).

Fig. 5. Effect of piroxicam and IL-1βRA on the phenylephrine induced ACTH and corticosterone secretion in control and stressed rats. +P<0.05 and ++P<0.01 vs. solvent-treated group; ^^P<0.01 vs. phenylephrine-treated control group; #P<0.01 vs. phenylephrine-treated stressed group.
Catecholaminergic and $\alpha_1$-adrenoceptors innervation of the PVN is required for full expression of the ACTH response to several stressors (23). Adrenergic $\alpha_1$-receptors mediate the release of arachidonic acid induced by activated phospholipase $A_2$ which regulates the synthesis of eicosanoids from neuronal membrane phospholipids. COX-1 and COX-2 isoenzymes generating prostaglandins may interfere with this synthesis during $\alpha_1$-adrenergic receptor stimulation (24).

Under physiological or pathological conditions different kinds of stressful stimuli, psychosocial-crowding stress activate the main stress regulatory center, the hypothalamic PVN via $\alpha$-adrenergic and cholinergic systems (25, 26). In rats crowded for 7 days piroxicam (0.2 mg/kg i.p.) given 15 min earlier significantly diminished the phenylephrine-induced ACTH (-45%) and corticosterone (-14%) secretion. This diminution was comparable with that induced in stressed rats by piroxicam (0.2 $\mu$g) in phenylephrine (30 $\mu$g) treated rats, when both drugs were administered i.c.v. (-55% and -26%, respectively) (4).

In stressed rats COX-2 inhibitor, compound NS-398 (0.2 mg/kg i.p.) moderately decreased the phenylephrine (0.4 mg/kg i.p.)-induced ACTH secretion (-25.3%) and did not substantially affect corticosterone secretion (+10.9%). These results suggest that inhibition of inducible COX-2 isoenzyme did not significantly alter the phenylephrine-induced ACTH and corticosterone secretion under basal or stress conditions when both these drugs were administered by either i.c.v. or i.p. route.

Interleukin-1$\beta$ receptor antagonist is known to be effective in antagonizing the IL-1$\beta$ receptors (5, 10, 27). In the present experiment IL-1$\beta$ receptor antagonist (5-50 $\mu$g/kg) given i.p. to non-stressed rats did not significantly alter the resting plasma ACTH and serum corticosterone levels. IL-1$\beta$ receptor antagonist given i.p. 15 min earlier moderately altered the i.p. phenylephrine (0.4 mg/kg)-induced ACTH and corticosterone secretion. These results suggest that IL-1$\beta$ is not significantly involved in mediation by PG of the phenylephrine-induced ACTH and corticosterone secretion under basal conditions in rats. This assumptions is corroborated by the fact that IL-1$\beta$ receptor antagonist (50 $\mu$g/kg) did not significantly alter the phenylephrine-induced ACTH and corticosterone response. Some reports also indicate a lack of inhibition by IL-1 receptor antagonist of IL-1$\beta$-induced-HPA axis response (7). In rats under basal conditions IL-1$\beta$ receptor antagonist (50 $\mu$g/kg i.p.) markedly enhanced the piroxicam-induced inhibition of the corticosterone response to phenylephrine. This may result from a direct stimulatory action of adrenergic agonist (28, 29) and IL-1$\beta$ (30) on glucocortical secretion from the rat adrenal cortex. In rats crowded for 7 days IL-1$\beta$ receptor antagonist (50 $\mu$g/kg i.p.) resulted in a stronger inhibition of the phenylephrine-induced ACTH secretion (39.8%) than piroxicam (2 mg/kg i.p.), a COX-1-inhibitor (14%). A combined pretreatment with IL-1$\beta$ receptor antagonist and piroxicam induced an additive inhibitory effect on the phenylephrine-induced ACTH secretion (-53.8%). Likewise, in stressed rats IL-1$\beta$ receptor antagonist
induced a much stronger reduction (-66.7%) of the phenylephrine-induced corticosterone response than piroxicam (-35.5%), a preferential COX-1 inhibitor. Combined treatment with both these drugs elicited an additive inhibition (-87%) of the phenylephrine-induced corticosterone secretion in stressed rats. IL-1β (2.5 µg/kg i.p.) considerably raised plasma ACTH and serum corticosterone levels in control rats (31) and crowding stress for 7 days significantly impaired these IL-1β-induced responses. Prostaglandins play a major role in the response of the hypothalamic-pituitary-adrenal axis to systemic IL-1β administration (32). The present results indicate a significant involvement of IL-1β in the HPA axis adrenergic stimulation in rats exposed to prolonged social stress. Under basal conditions IL-1β does not seem to play a significant role in the α1-adrenergic receptor stimulation of HPA axis.

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