THE INFLUENCE OF INDOMETHACIN ON THE ACTH SECRETION INDUCED BY CENTRAL STIMULATION OF ADRENERGIC RECEPTORS

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We had previously demonstrated that indomethacin affected the corticosterone secretion induced by central stimulation of α-but not β-adrenergic receptors in conscious rats. In the present study we investigated whether hypothalamic and/or pituitary prostaglandins (PGs) were involved in the central adrenergic stimulation of ACTH secretion. Indomethacin, 2 mg/kg ip or 10 μg intracerebroventricularly (icv), was administered 15 min before phenylephrine (30 μg icv), an α1-adrenergic agonist, clonidine (10 μg), an α2-adrenergic agonist, and isoprenaline (20 μg) or clenbuterol (10 μg), a β1- or β2-adrenergic agonist. One hour after the last injection the rats were decapitated and plasma levels of ACTH were measured. The present results show that the ACTH responses induced by icv administration of phenylephrine and clonidine were considerably impaired by icv or ip pretreatment with indomethacin, an inhibitor of prostaglandin synthesis. Indomethacin given by either route only slightly diminished the isoprenaline-induced ACTH response and did not substantially alter the clenbuterol-induced response. The adrenergic-induced ACTH responses were more potently inhibited by ip than by icv pretreatment with indomethacin, which may result from a stronger inhibition of PGs synthesis in the median eminence and anterior pituitary by ip pretreatment with indomethacin than in hypothalamic structures by its icv administration. These results indicate a significant involvement of PGs in central stimulation of the hypothalamic-pituitary adrenal (HPA) axis by α1- and α2- but not β-adrenergic receptors.

Key words: central adrenergic receptors, indomethacin, prostaglandins, ACTH secretion.

INTRODUCTION

It is generally recognized that the central adrenergic system participates in regulation of the hypothalamic-pituitary-adrenal axis under basal and stress circumstances (1—4). Intracerebroventricular administration of adrenergic receptors agonists, via α1-, α2- and β-adrenergic receptor, stimulates corticotropin-releasing hormone (CRH) secretion from the hypothalamic...
paraventricular nucleus (PVN) and the secretion of ACTH from the anterior pituitary corticotrophs (3, 5, 6).

Some earlier studies suggested that sympathetic nerve stimulation or administration of adrenergic agonists increased the synthesis of prostaglandins in various tissues and could be involved in modulation of eicosanoid metabolism. Neurotransmitters, particularly noradrenaline, dopamine and acetylcholine, can modulate the arachidonic acid cyclooxygenase pathway (7) and influence prostanoid synthesis. Noradrenaline enhances incorporation of arachidonic acid into phosphatidylinositol and induces PGE₂ release in various rodent brain areas (8). However, involvement of particular adrenergic receptors in the formation and release of PGs is still unclear. In some peripheral tissues, such as rabbit aorta and rat kidney, the synthesis is linked to α-adrenergic receptors (9, 10). Venous α₁- and α₂-adrenergic stimulation by phenylephrine and clonidine releases vasodilator PGs that antagonize the venoconstrictor response (11). Prostaglandin synthesis elicited by an adrenergic transmitter in the heart is mediated by activation of β-adrenergic receptors (12). In the central nervous system the inhibition of prostaglandin-induced hyperalgesia by indomethacin is mediated by α-adrenoceptors since it is blocked by phenotolamine (13).

The ACTH release induced by noradrenaline in rats is mediated by prostaglandin E₂, since iv injection of noradrenaline produces dose-dependent increases in plasma concentrations of ACTH and PGE₂, while indomethacin, an inhibitor of PG synthesis, significantly suppresses this increase in plasma ACTH (14). Although prostaglandin E₂ is considered to be the most important PG in the brain that mediates the cytokine-induced ACTH secretion, also PGE₁ and PGE₂α may mediate this stimulatory action (15). We found that indomethacin considerably impaired the corticosterone response to icv administration of phenylephrine and clonidine, an α₁- and α₂-receptor agonist, respectively (16, 17). However, pretreatment with indomethacin did not markedly affect the increase in corticosterone secretion elicited by icv isoprenaline, a β-adrenergic receptor agonist.

Although our previous studies may suggest that indomethacin induces similar alterations in the adrenergic agonists-induced CRH and ACTH secretion, it is not known whether and to what extend PGs at the hypothalamic and/or pituitary level mediate the ACTH secretion evoked by icv administration of adrenergic receptor agonists. Therefore the purpose of the present experiment was to investigate the involvement of PGs in ACTH secretion stimulated centrally by adrenergic receptor agonists in conscious rats. In addition, by icv or ip administration of indomethacin before adrenergic agonists, possible participation of PGs at the hypothalamic, pituitary, and/or suprarenal gland levels was determined. Also putative involvement of PGs in the central activation of the HPA axis by clenbuterol, a selective β₂-adrenergic receptor agonist, and a more potent stimulator of anterior pituitary secretion than isoprenaline, was investigated.
MATERIALS AND METHODS

Animals

Male Wistar rats weighing 190—230 g were housed in cages at a room temperature of 20±2°C and on a daylight cycle at least one week before the experiment. Standard laboratory food and tap water were provided ad libitum. For intracerebroventricular injections, the skulls of rats were prepared one day earlier under light ether anesthesia. The rats remained in their home cages until they were scheduled for treatment. Institutional Bioethical Committee approved the study.

Experiments

The rats were randomly assigned to one of the experimental groups (6 animals each). In four experimental groups, the rats were injected into the right cerebral ventricle with adrenergic agonists contained in 10 µl of saline: phenylephrine (30 µg), clonidine (10 µg), isoprenaline (20 µg) and clenbuterol (10 µg), or with indomethacin (2 mg/kg ip or 10 µg icv) 15 min before each adrenergic agonist. Indomethacin was dissolved in a 4% sodium bicarbonate at a concentration of 1 mg/ml. Control rats received simultaneously the same volume of saline or solvent. One hour after the last injection, the rats were decapitated immediately after their removal from the cage and their trunk blood was collected. Control rats were decapitated concurrently with the experimental group to obtain resting plasma ACTH levels.

In order to avoid interference with circadian rhythm in ACTH levels, all experiments were performed between 9 and 11 a.m. and all decapitations were carried out between 10 and 12 a.m., i.e. when plasma hormone levels are low in normal circadian rhythm.

ACTH determinations

Trunk blood samples were collected on ice in conical plastic tubes containing 200 µl of a solution of EDTA, 5 mg/ml, and aprotinin, 500 TIU (Sigma). 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 a double antibody 125I radioimmunoassay obtained from CIS Bio International, and were calculated as pg/ml of the plasma. One analysis was performed in each rat’s plasma, but 6 animals were used in each experimental group.

Drugs

The following drugs were used: L-phenylephrine hydrochloride, DL-isoproterenol hydrochloride, clenbuterol hydrochloride, indomethacin (Sigma) and clonidine (Boehringer). Indomethacin was dissolved in a 4% sodium bicarbonate and the remaining drugs were dissolved in sterile saline immediately before use; the doses used are expressed in terms of salts.

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 comparisons with Duncan’s test. The results were considered to be significantly different when p<0.05.
RESULTS

Effect of indomethacin on basal ACTH level

Due to limited penetration of brain structures by indomethacin from peripheral circulation, this cyclooxygenase blocker was given intraperitoneally (2 mg/kg) or intracerebroventricularly (10 μg). Given alone by either route, indomethacin slightly decreased the basal plasma ACTH level 1 h after administration (Table 1). The indomethacin-induced decrease in basal plasma ACTH level was identical after both ip and icv administration. That observation may suggest a minor inhibitory effect of indomethacin on basal ACTH release from anterior pituitary corticotrophs.

Table 1. Effect of indomethacin on basal plasma ACTH level

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ACTH pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>60.5 ± 3.5</td>
</tr>
<tr>
<td>Indomethacin 2 mg/kg ip</td>
<td>41.0 ± 6.0</td>
</tr>
<tr>
<td>Indomethacin 10 μg icv</td>
<td>41.0 ± 8.0</td>
</tr>
</tbody>
</table>

Indomethacin was injected 1 h before decapitation. Values represent the mean ± SEM of 6 rats.

Inhibition by indomethacin of phenylephrine-induced ACTH secretion

Phenylephrine (30 μg), an α₁-adrenergic receptor agonist, given icv induced a significant increase in ACTH secretion measured 1 h after administration. Systemic pretreatment with indomethacin (2 mg/kg) 15 min prior to phenylephrine considerably diminished, by 50%，the phenylephrine-induced ACTH secretion. Intraventricular pretreatment with indomethacin (10 μg) also significantly decreased, by 38%, the phenylephrine-induced ACTH secretion (Fig. 1).

Diminution by indomethacin of clonidine-induced ACTH secretion

Intraventricular administration of clonidine (10 μg), an α₂-adrenergic receptor agonist, significantly increased plasma ACTH level 1 h after administration. Intraperitoneal pretreatment with indomethacin (2 mg/kg) considerably impaired, by 67%, the clonidine-induced ACTH secretion. Intraventricular pretreatment with indomethacin (10 μg) was somewhat less effective than ip pretreatment in decreasing the clonidine-elicited pituitary-adrenocortical activity, though it also significantly diminished, by 45%, the secretion of ACTH (Fig. 2).
Fig. 1. Effect of indomethacin on the phenylephrine-induced plasma ACTH levels. Indomethacin was injected ip or icv 15 min before icv phenylephrine. One hour after the last injection the rats were decapitated. Values represent the mean ±SEM of 6 rats. ++p < 0.05 vs. saline control and **p < 0.05 vs. adrenergic agonist treated group.

Fig. 2. Effect of indomethacin on clonidine-induced plasma ACTH levels. Indomethacin was injected ip or icv 15 min before icv clonidine. One hour after the last injection the rats were decapitated. Values represent the mean ±SEM of 6 rats. ++p < 0.05 vs. saline control and **p < 0.05 vs. adrenergic agonist treated group.
Effect of indomethacin on isoprenaline-induced ACTH secretion

Intracerebroventricular administration of isoprenaline (20 μg), a nonselective β-adrenergic receptor agonist, considerably augmented the secretion of ACTH measured 1 h after administration. Systemic pretreatment with indomethacin (2 mg/kg ip) moderately diminished, by 37%, the isoprenaline-induced ACTH response. Intraventricular pretreatment with indomethacin induced a minor, 25%, reduction of ACTH secretion. The inhibitory effect of indomethacin, given by either route, on the isoprenaline-induced hormone secretion were not statistically significant (Fig. 3).

![Graph showing ACTH levels](image)

**Fig. 3.** Effect of indomethacin on isoprenaline-induced plasma ACTH levels. Indomethacin was injected ip or icv 15 min before icv isoprenaline. One hour after the last injection the rats were decapitated. Values represent the mean ± SEM of 6 rats. ++p < 0.05 vs. saline treated group.

Effect of indomethacin on clenbuterol-induced ACTH secretion

Clenbuterol (10 μg), a selective β₂-adrenergic receptor agonist, given icv stimulated ACTH secretion more potently than isoprenaline. One hour after administration, clenbuterol raised plasma ACTH level from the control value of 72 up to 1291 pg/ml compared to 598 pg/ml after isoprenaline administration. Systemic pretreatment with indomethacin (2 mg/kg) slightly lowered, by 27%, while icv pretreatment did not alter the clenbuterol-induced ACTH response (Fig. 4).
Fig. 4. Effect of indomethacin on clenbuterol-induced plasma ACTH levels. Indomethacin was injected ip or icv 15 min before icv clenbuterol. One hour after the last injection the rats were decapitated. Values represent the mean ± SEM of 6 rats. ++p < 0.05 vs saline treated group.

DISCUSSION

The present study shows that the adrenergic α₁- and β₁-receptor agonists, phenylephrine and clonidine, administered intracerebroventricularly elicit considerable stimulation of ACTH; likewise, they increased corticosterone secretion in our previous experiments (16, 17). These agonists and catecholamines are known to stimulate CRH from rat hypothalamic explants via adrenergic receptors (1). In the present experiments isoprenaline, a nonselective β-adrenergic receptor agonist, and clenbuterol, a specific β₂-receptor agonist, induced more potent stimulation of ACTH secretion, than phenylephrine and clonidine, an α₁- and β₂-adrenergic receptor agonist, respectively. This finding contrasts with a weaker CRH response to isoproterenol than to phenylephrine and clonidine, observed in hypothalamic explants in vitro (1) and cannot be satisfactorily explained.

Our study shows involvement of PGs in central α-adrenergic receptor stimulation of the HPA axis. Indomethacin, a non-selective cyclooxygenase inhibitor, given ip or icv 15 min prior to phenylephrine significantly diminished the phenylephrine-induced ACTH secretion. That diminution was more potent after ip than icv pretreatment with indomethacin; the respective decreases in plasma ACTH levels were 50 and 38%. Indomethacin also significantly
impaired the clonidine-induced ACTH response. Given ip indomethacin elicited a stronger decline in ACTH secretion (by 67%) than when it was administered icv (by 45%). Both α₁- and α₂-adrenergic receptors are present in the hypothalamic PVN therefore they may mediate the release of CRH after icv administration of phenylephrine and clonidine. These receptors are also involved in the stimulatory effect of noradrenaline on CRH release from the incubated or superfused mediobasal hypothalamus (6).

Indomethacin given icv may penetrate hypothalamic CRH neurons and inhibit the PGs synthesis induced by central administration of adrenergic agonists. Noradrenaline given intravenously is known to increase the plasma concentration of both ACTH and prostaglandin E₂ (14). Prostaglandin E₂ is also involved in the interleukin-1β-induced CRH-and ACTH secretion in rats (18, 19). Indomethacin poorly penetrates the blood-brain barrier from systemic circulation, but it may reach hypothalamic CRH neurons through the organum vasculosum of the lamina terminalis, a site almost devoid of the blood-brain barrier (20). However, such penetration is limited, hence it seems unlikely that in the present experiment ip administered indomethacin could significantly influence the adrenergic agonist-inducted CRH release from the hypothalamic paraventricular nucleus. Indomethacin given systemically reaches easily the hypothalamic median eminence (ME) and anterior pituitary, situated outside the blood-brain barrier. When given systemically, indomethacin is able to reduce the ACTH response evoked by IL-1β injected into the hypothalamic ME (19) or intraarterially, which indicates that PG-dependent mechanism conveys IL-1β signals to effect the HPA axis response (21). Systemically administered indomethacin may also inhibit the PGs synthesis in anterior pituitary corticotrophs and impair the secretion of ACTH. The extent of direct inhibition of ACTH secretion from anterior pituitary by indomethacin is not known.

Isoprenaline, a nonselective β-adrenergic receptor agonist and clenbuterol, a long-acting β₂-adrenoceptor selective agonist, given icv considerably increased ACTH secretion. clenbuterol was more potent than isoprenaline in this respect, indicating predominant involvement of central β₂-adrenergic receptors in the HPA stimulation. Isoprenaline has equal affinity for β₁- and β₂-adrenoceptors and is a full agonist at both these receptor subtypes. However, it induces a preferential action on β₂-adrenoceptors in the rat brain in vivo (22), due to tonic regulation of β₁- but not β₂-adrenoceptors by the endogenously released noradrenaline, which may cause β₁-adrenoceptors less sensitive to the exposure to endogenous agonists. The presence of β-adrenoceptors has been found on majority of the ACTH containing pituitary corticotrophs (5). After icv administration isoprenaline and clenbuterol can reach the hypothalamic PVN and activate β-adrenoceptors to stimulate the release of CRH,
which reaches the anterior pituitary corticotrophs and enhances ACTH secretion. In the present experiment icv or ip pretreatment with indomethacin slightly diminished the isoprenaline- and the clenbuterol-elicited ACTH response. A moderate inhibition by indomethacin of the isoprenaline-induced ACTH response in the present experiment may in part depend on IL-1β, which can be induced by icv isoprenaline (23), and is known to stimulate ACTH secretion by PG mediation (18, 21, 24, 25). These data suggest that the isoprenaline-induced activation of the HPA axis may partly depend on PG synthesis. Our findings indicate that central β-adrenergic stimulation of HPA axis does not involve marked involvement of prostaglandins.

Prostaglandins generated by icv administration of adrenergic agonists can directly stimulate CRH release from the hypothalamic PVN, since intrahypothalamic injection of PGE₂ stimulates the secretion of CRH and ACTH (18). Although adrenergic agonists administered icv stimulate CRH neurons in the hypothalamic PVN, systemic indomethacin probably acts mainly at the level of CRH and adrenergic terminals in the median eminence situated outside the blood-brain barrier. In the present experiment the increased ACTH secretion evoked by all adrenergic agonists used was more potently inhibited by ip than icv pretreatment with indomethacin. Indomethacin given ip is also likely to inhibit prostaglandin synthesis in the anterior pituitary which contains adrenergic receptors and is readily accessible from the systemic circulation. It seems unlikely that prostaglandins centrally generated by icv adrenergic receptor agonists reach, via portal circulation, anterior pituitary in sufficient concentrations to affect markedly ACTH secretion.

The effect of indomethacin on ACTH secretion evoked by central stimulation of adrenergic receptors resembles the action of nitric oxide synthase blocker L-NAME in our recent study (26). This blocker evoked also similar reduction in ACTH secretion induced by α₁- and α₂-adrenergic receptor stimulation. These findings suggest that adrenergic agonists given icv may induce both cyclooxygenase and nitric oxide synthase with increases in the release of PGs and NO in central structures involved in HPA axis stimulation. An interaction between NO and cyclooxygenase pathways has been proposed in different regulations. However, such an interaction in stimulation the HPA axis activity is not known.

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