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INFLUENCE OF CYCLOOXYGENASE INHIBITORS ON THE CENTRAL HISTAMINERGIC STIMULATION OF HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

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Brain histamine participates in central regulation of the hypothalamic-pituitary-adrenal (HPA) axis. Endogenous prostaglandins modulate signal transduction of different neurotransmitters involved in activation of HPA axis. In the present experiment we investigated whether endogenous prostaglandins are involved in the stimulation of ACTH and corticosterone secretion by histaminergic systems in the rat brain. Histamine (50 μg), histamine-trifluoromethyl-toluidine derivative (HTMT, 75 μg) a selective and potent H_1-receptor agonist, and amthamine (50 μg) a H_2-receptor agonist given intracerebroventricularly (i.c.v.) to non-anesthetized rats considerably increased ACTH and corticosterone secretion 1h after administration. A non-selective cyclooxygenase inhibitor indomethacin (2 mg/kg i.p. or 10 μg i.c.v.), piroxicam (0.02 and 0.2 μg i.c.v.) a more potent antagonist of constitutive cyclooxygenase (COX-1) and compound NS-398 (0.1 and 1.0 μg i.c.v.), a selective inhibitor of inducible cyclooxygenase (COX-2) were given 15 min before histamine and histamine receptor agonists. One hour after the last injection trunk blood from decapitated rats was collected for hormones determination. The histamine-induced ACTH and corticosterone secretion was significantly diminished by piroxicam and was not markedly altered by indomethacin and compound NS-398. The HTMT-elicited increase in ACTH and corticosterone secretion was significantly prevented by indomethacin and was not affected by piroxicam or compound NS-398. The amthamine-evoked increase in ACTH and corticosterone secretion was not markedly influenced by any cyclooxygenase blocker applied in the present experiment. These results indicate that the histamine H_1-receptor transmitted central stimulation of the HPA axis is considerably mediated by prostaglandins generated by consititutive cyclooxygenase, whereas stimulation transmitted *via* H_2-receptor does not significantly depend on endogenous prostaglandins mediation.

Key words: *histamine, HTMT, amthamine, prostaglandins, COX-1, COX-2, ACTH, corticosterone.*

INTRODUCTION

It is well known that diffuse histaminergic nerve fibres consisting of long varicose, arborizing, slowly conducting axons project from tuberomammillary nucleus to virtually all parts of the brain. These axons form synaptic contacts with other neurons and varicosities approximate to neurones and to glial cells and capillaries (1, 2). The highest density of histamine fibres has been found in the ventral half of the posterior hypothalamus, the median eminence and in the suprachiasmatic and paraventricular nuclei. All areas of the hypothalamus receive a moderate to strong histaminergic innervation. Histamine actions in the hypothalamus provide a cellular correlate for its effects on drinking, eating, body temperature, circadian rhythms and hormone release. Histamine influences the release of many hormones from the pituitary gland *via* an action at the hypothalamic level. Central histamine has a stimulatory action on the release of the proopiomelanocortin (POMC)-derived peptides ACTH, β-endorphin, as well as on the release of vasopressin, oxytocin and prolactin. The action of histamine on the POMC-derived peptides is not due to an action on the pituitary gland but is indirect, involving a stimulation of corticotropin releasing hormone (CRH) in the paraventricular nucleus (PVN) and vasopressin releasing cells in the supraoptic nucleus (3). Many brain nuclei are excited *via* histamine H_1 receptors including the supraoptic, vasopressin neurons. Histaminergic system in the brain is known to stimulate the HPA axis through three pharmacologically distinct receptor subtypes, termed H_1, H_2 and H_3 (4-6). High densities of H_1 receptors are present in the limbic system, including many nuclei of the hypothalamus. Like the histamine H_1 receptor, the H_2 receptor has a widespread expression in the brain. In contrast to H_1 receptors, H_2 receptors are present in low densities in hypothalamic nuclei (7).

Histamine H_1 receptor belongs to the superfamily of receptors coupled to G-proteins. Stimulation of H_1-receptor releases inositol phosphate and can lead to the formation of arachidonic acid, most likely through the activation of phospholipase A_2 and to the formation of cGMP (4). A single H_2 receptor may be linked not only to adenylyl cyclase activation but also to reduction of phospholipase A_2 activity. Prostaglandins (PG) modulate the release of neurotransmitters as well as several hormones of the HPA axis. Prostaglandins can also amplify neurotransmitter-mediated signals (8). Previous studies have shown that central administration of PG stimulates ACTH secretion probably via activation of CRH neurons in the hypothalamic paraventricular nuclei (9). Multiple PG may be involved in the HPA axis stimulation in rodents (10). We have found that central PG are involved in the HPA axis stimulation by adrenergic and cholinergic agonists and constitutive and inducible cyclooxygenase participate, to different extent, in the stimulation of ACTH and corticosterone secretion (11, 12). It is not clear whether endogenous prostaglandins generated by constitutive and inducible cyclooxygenase (COX-1 and COX-2) mediate the HPA

axis response stimulated *via* histamine H_1 and H_2 receptors. These receptors are known to be involved in the histamine-induced ACTH and corticosterone release (13).

The objective of the present study was to investigate a possible role of prostaglandins generated by COX-1 and COX-2 in the central stimulation of HPA axis by histamine and histamine receptor agonists in non-anesthetized rats.

MATERIALS AND METHODS

Animals

The experiments were carried out on Male Wistar rats, weighing 180-200 g, housed in groups of 6-8 per cage with free access to food and water. The animals were kept under a 12/12 h light/dark cycle, light on at 7 a.m. at an ambient room temperature of 19-21°C, one week prior to experimentation. Experimental protocols were approved by the local Ethics Committee. For intracerebroventricular injections, the skulls of rats were prepared one day earlier under light ether anesthesia for free-hand i.c.v. injections. The rats remained in their home cages until they were scheduled for treatment.

Experiments

The rats were randomly assigned to one of the experimental groups (6 animal each). In the first group control rats were injected with 10 µl of saline or respective vehicle into the right cerebral ventricle. Also the effect of cyclooxygenase blockers alone on the basal plasma ACTH and serum corticosterone levels in control rats were determined. In the second group rats were injected i.c.v. with histaminergic agonists contained in 10 µl of solvent: histamine (50 µg) , histamine-trifluoromethyl-toluidine (HTMT 75 µg), a histamine H_1-receptor agonist, and amthamine (50 µg), a histamine H_2-receptor agonist. The histaminergic agents were used in doses which resulted in a considerable increase in ACTH and corticosterone secretion 1h after administration in our earlier study (14). In the third group cyclooxygenase inhibitors were administered i.c.v., piroxicam and compound NS-398 and indomethacin which was given also by i.p. route 15 min prior to histamine and histamine receptor agonists. 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 groups to obtain basal plasma ACTH and serum corticosterone levels. In order to avoid interference with the circadian rhythm in ACTH and corticosterone levels, all experiments were performed between 9 and 11 a.m. and all decapitations were carried out between 10 and 11 a.m. i.e. when plasma hormone levels are low in a normal diurnal rhythm.

ACTH and corticosterone determinations

Trunk blood samples were collected on ice in conical plastic tubes containing 200 µl of a solution of EDTA, 5mg/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 ^{125}I radioimmunoassay obtained from CIS Bio International, and were calculated as pg/ml of the plasma. The concentration of corticosterone was measured fluorometrically and expressed as

μg/100 ml. One analysis was performed in each rat`s plasma, but 6 animals were used for each data point.

Drugs

The following drugs were used: histamine hydrochloride (Sigma), HTMT dimaleate (6-[2-(4-imidazolyl)etyloamino]-N-(4-trifluoromethylphenyl)heptanecarboxamide), amthamine hydrochloride (2-amino-5-(2-aminoethyl)-4-methylthiazole (Torcis Cookson) and indomethacin, and piroxicam were purchased from Sigma and compound NS-398 from Cayman Chemical Co (Ann Arbor Mi, USA). Histamine and amthamine were dissolved in a sterile saline, HTMT was dissolved in concentrated ethanol and diluted with sterile water and indomethacin and piroxicam were prepared for injection by sonification in 1% Tween solution. All drug solutions were prepared immediately before use; the doses used are expressed in terms of salts. The required doses of drugs were injected i.c.v. in 10 μl of solvent and 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 comparisons with Duncan`s test. The results were considered significantly different when $p<0.05$.

RESULTS

The vehicles used for dissolving the compounds given alone in respective volumes i.c.v. or i.p. did not substantially alter basal plasma ACTH and serum corticosterone levels 1h later. Likewise, cyclooxygenase inhibitors indomethacin, piroxicam and compound NS-398 alone, given i.c.v. or i.p. in doses used in the present study did not affect basal ACTH and corticosterone levels.

Effect of cyclooxygenase antagonists on histamine-induced ACTH and corticosterone secretion

Histamine (50 μg) administered i.c.v. 1h after injection induced a significant increase in plasma ACTH and serum corticosterone levels, from the control level of 121 to 1303 pg/ml and from 7.1 to 47.4 μg/dl, respectively. Indomethacin, a non-selective COX blocker, given either i.c.v. (10 μg) or i.p. (2 mg/kg) 15 min prior to histamine did not substantially alter the i.c.v. histamine-induced ACTH and corticosterone response. The alterations induced by indomethacin (from -8.7 to +20%) were not statistically significant. Pretreatment of rats with piroxicam (0.2 μg i.c.v.) a more potent COX-1 blocker than indomethacin, significantly diminished both the histamine-induced ACTH response, by 29.3% and corticosterone response by 19.6%. Pretreatment of rats with a selective COX-2 inhibitor, compound NS-398 (0.1 or 1.0 μg i.c.v.), did not markedly alter the histamine-evoked increase in ACTH and corticosterone secretion (*Fig. 1*).

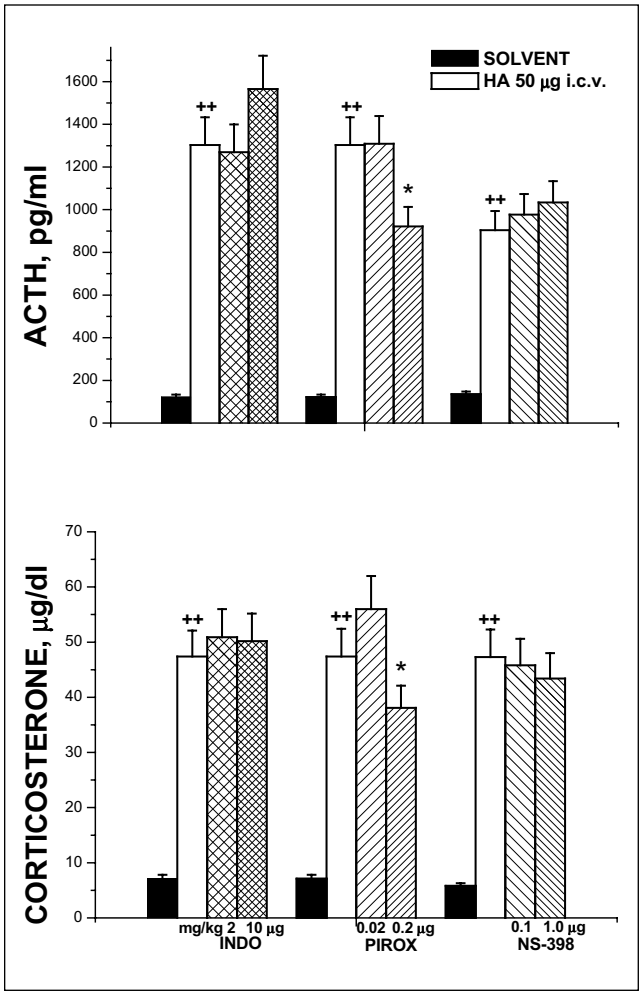

Fig. 1. Effect of COX blockers: indomethacin, piroxicam and compound NS-398 on the histamine (HA)-induced plasma ACTH and corticosterone levels. In *Fig. 1-3* COX blockers were injected i.c.v., indomethacin was also injected i.p., 15 min before i.c.v. histamine or histamine agonist and 1h after the last injection the rats were decapitated. In *Fig 1-3* values represent the mean ± SEM of 6 rats. ++p <0.01 vs. solvent controls and *p<0.05 vs. histamine or histamine receptor agonist-treated group.

Effect of cyclooxygenase blockers on HTMT-induced ACTH and corticosterone response

Histamine H_1-receptor agonist, HTMT (75 µg) given i.c.v. induced 1h later an increase of plasma ACTH level from 87.2 to 561.6 pg/ml and corticosterone level from 8.6 to 35.1 µg/dl. This significant stimulatory effect, however, was markedly lower than the effect of histamine. Systemic pretreatment with indomethacin (2 mg/kg i.p.) considerably impaired the ACTH and corticosterone response to HTMT given 15 min later, by 48.5% and 27.9%, respectively. Also intraventricular pretreatment with indomethacin (10 µg) significantly diminished the HTMT-induced ACTH response (by 28.4%) but did not affect corticosterone response. Piroxicam (0.02 and 0.2 µg i.c.v.), a COX-1 antagonist, slightly

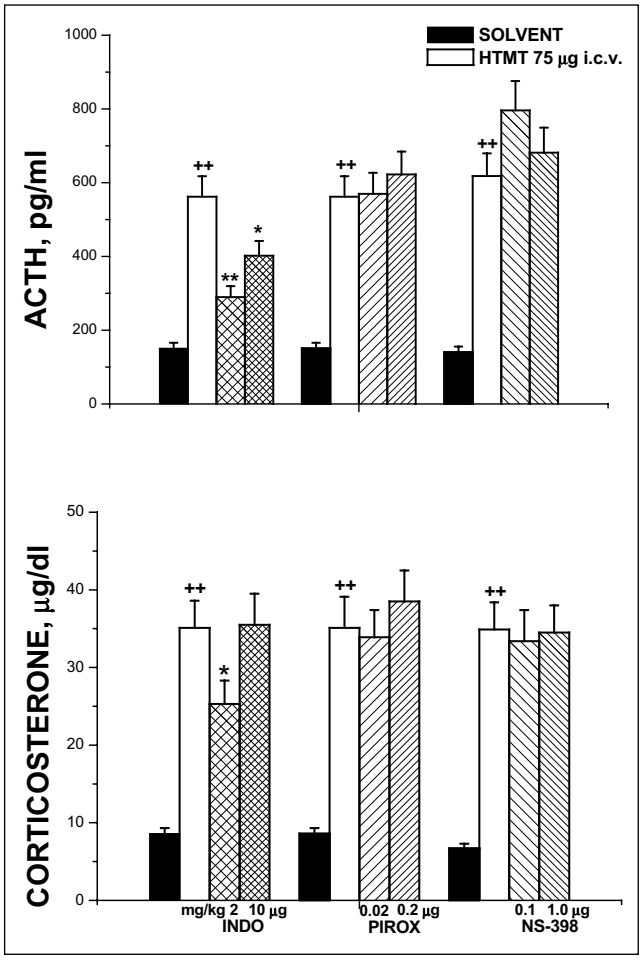

Fig. 2. Effect of indomethacin, piroxicam and compound NS-398 on the histamine-trifluoromethyl-toluidine (HTMT)-induced plasma ACTH and corticosterone levels. See legend to *Fig. 1*.

augmented the HTMT elicited increase in ACTH and corticosterone secretion, by 10.8 and 9.7%, ($p>0.05$) respectively (*Fig. 2*). Pretreatment with compound NS-398 a selective COX-2 inhibitor, (0.1 and 1.0 µg i.c.v.) moderately increased the HTMT-induced ACTH response up to 28.7% and did not alter corticosterone response. Therefore neither COX-1 nor COX-2 blocker induced any marked changes in the HTMT-elicited hormone levels (*Fig. 2*).

Effect of cyclooxygenase blockers on AMT-induced ACTH and corticosterone levels

Amthamine (50 µg i.c.v.) considerably increased ACTH and corticosterone secretion 1h after its administration, from 141 to 958 pg/ml and from 5.4 to 57.3 µg/dl, respectively. Neither systemic nor i.c.v. pretreatment with indomethacin (2 mg/kg or 10 µg) substantially alter the AMT-induced ACTH and corticosterone

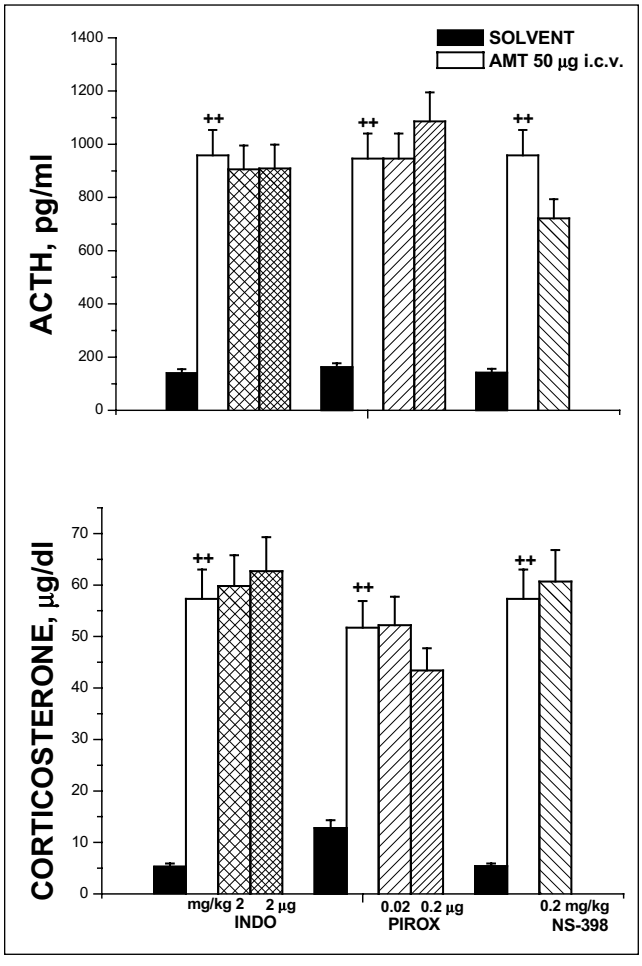

Fig. 3. Effect of indomethacin, piroxicam and compound NS-398 on the amthamine (AMT)-induced plasma ACTH and corticosterone levels. See legend to *Fig. 1*.

secretion. Likewise, pretreatment with piroxicam (0.02 and 0.2 µg i.c.v.), a COX-1 blocker, did not affect the AMT-evoked ACTH and corticosterone secretion (*Fig. 3*). In the present experiment compound NS-398, a COX-2 selective antagonist was given 0.2 mg/kg i.p. in a dose that was used to block COX-2-induced reactions. It moderately diminished the amthamine-induced ACTH response (by 24.7%) but did not affect corticosterone response (*Fig. 3*).

DISCUSSION

In the present experiment, like in our earlier study (14), i.c.v. histamine (50 µg), HTMT (75 µg), a histamine H_1-receptor agonist, and amthamine (50 µg), a histamine H_2-receptor agonist resulted in a considerable increase in ACTH

and corticosterone secretion 1h after administration. The most pronounced stimulatory effect was induced by histamine and markedly weaker effect was elicited by amthamine, a histamine H_2-receptor agonist and still weaker stimulation was evoked by HTMT, H_1-receptor agonist. Amthamine behaves as full agonist equipotent to, or slightly more active than histamine in various cardiovascular preparations. Since amthamine does not induce any significant functional effect on the H_1 receptor, it can be considered a potent and selective H_2 receptor agonist (6). In the stimulation of some cellular reactions amthamine was also reported to be a stronger agent than HTMT and histamine itself (15). Our present data on the order of potency of histamine, amthamine and HTMT in the stimulatory action of HPA axis agree with reported intensity of their action in different biological systems. The stimulatory effect of histamine on HPA axis is predominantly indirect, exerted by release of CRH in the hypothalamus, and histaminergic postsynaptic H_1 and H_2 receptors are involved in that stimulation (13).

In the present experiment indomethacin given i.p. (2 mg/kg) or i.c.v. (10 μg) did not evoke any significant effect on the histamine-induced increase in ACTH and corticosterone secretion while pretreatment with piroxicam (0.2 μg) a COX-1 blocker, significantly diminished the stimulatory effect of i.c.v. histamine on ACTH and corticosterone secretion. Although indomethacin does not readily penetrate the blood-brain barrier and tissues, it was able to considerably inhibit the HPA axis stimulation by adrenergic and cholinergic agonists in our earlier studies after both central and peripheral administration (12, 17). Histamine results in much stronger stimulatory effect on HPA axis by activation of H_2 receptors than by stimulation of H_1 receptors, which may explain a lack of inhibitory effect of indomethacin on the stimulation of HPA by histamine. Piroxicam, a more potent COX-1 blocker than indomethacin inhibited significantly the histamine-induced ACTH and corticosterone secretion. This result suggests that PG may mediate a part of central histamine-induced stimulation of HPA axis transmitted *via* H_1-receptors. Although COX-2 immunoreactive cells are localized in the PVN of the hypothalamus which suggests that COX-2 may be involved in processing of the endocrine responses (18), compound NS-398 (0.1 μg), a COX-2 blocker given prior to histamine did not markedly affect the histamine-induced ACTH and corticosterone secretion. The present results, therefore do not indicate any marked involvement of COX-2 in the histamine-induced HPA axis stimulation. This finding agrees with the accepted suggestion that a modulatory role of brain histamine is primarily mediated through histamine H_1 receptors (19).

In the present experiments the significantly increased HPA axis secretory activity by HTMT, a histamine H_1-receptor agonist, was considerably impaired by pretreatment with indomethacin a non-selective COX inhibitor, given i.p. or i.c.v. and by piroxicam (0.02 μg i.c.v.), a more potent COX-1 inhibitor than indomethacin. Histamine H_1 receptors have been reported to stimulate arachidonic acid release. Inhibition of its metabolic pathway by COX-1

antagonist - piroxicam and indomethacin may account for the significant inhibition of the HTMT-elicited secretion of ACTH and corticosterone (20). In our experiment compound NS-398 (0.1 μg), a selective COX-2 inhibitor, moderately augmented the HTMT-induced increase of ACTH secretion and did not affect corticosterone secretion. This finding may suggest, that in the HTMT-induced increase in ACTH and corticosterone secretion PG generated by COX-2 are not significantly involved. Moreover, histamine H_2 receptors may inhibit the release of arachidonic acid stimulated by H_1 receptors and may account for the opposite effects elicited by these two receptor subtypes during stimulation of HPA axis (20, 21).

Stimulatory effect of AMT, a H_2-receptor agonist, was not significantly altered by pretreatment with either i.p. or i.c.v. indomethacin. Some decrease of plasma ACTH levels and increase in serum corticosterone levels were neither significant nor dose-dependent in comparison with the AMT-induced levels. Likewise, neither piroxicam nor compound NS-398 induced any marked alteration of the AMT-induced plasma ACTH and serum corticosterone levels. In addition, COX-2 blocker was unable to substantially affect the HPA axis response to histamine or H_1 and H_2 agonist in the present experiment.

These results, therefore, do not suggest any marked mediation of endogenous PG in the HPA activity stimulated *via* central histamine H_2 receptors. The obtained results clearly suggest the involvement of prostaglandins generated by constitutive cyclooxygenase in the stimulation of HPA axis by histamine H_1 receptor.

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