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## INVOLVEMENT OF NITRIC OXIDE IN CENTRAL HISTAMINERGIC STIMULATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

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Brain histamine participates in central regulation of the hypothalamic-pituitary-adrenal (HPA) axis. Endogenous nitric oxide (NO) modulates signal transduction of some neurotransmitters involved in activation of the HPA axis. In the present study we investigated whether endogenous NO and histaminergic systems in the rat brain interact in their regulation of ACTH and corticosterone secretion. Histamine (50 µg), histamine-trifluoromethyl-toluidide derivative (HTMT, 75 µg) a selective and potent H<sub>1</sub>-receptor agonist, and amthamine (75 µg) a H<sub>2</sub>-receptor agonist given intracerebroventricularly (i.c.v.) considerably increased ACTH and corticosterone secretion 1 h after administration. A potent and competitive inhibitor of rat brain neuronal NO synthase, (NOS), 7-nitroindazole (7-NI), given i.p. 15 min before histamine moderately increased the histamine-induced ACTH secretion and did not substantially alter the histamine-induced corticosterone secretion. Pretreatment with 7-NI totally abolished the HTMT-induced increase in ACTH and corticosterone secretion. The amthamine-evoked rise in ACTH secretion was moderately diminished and the amthamine-induced corticosterone secretion was not substantially altered by pretreatment with 7-NI. These results suggest that the histamine H<sub>1</sub>-receptor transmitted central stimulation of the HPA axis is considerably mediated by endogenous NO, whereas stimulation by histamine and via H<sub>2</sub>-receptor does not significantly depend on endogenous NO mediation.

**Key words:** *histamine, HTMT, amthamine, nitric oxide, ACTH, corticosterone.*

## INTRODUCTION

Histamine, the aminergic neurotransmitter in the mammalian central nervous system, plays an important role in central neuroendocrine regulations. Histamine does not easily cross the blood-brain barrier, therefore histamine receptors in the central nervous system mediate the actions of locally

synthesized amine (1). Histamine evidently affects the secretion of ACTH and corticosterone and seems indispensable to the normal functioning of the hypothalamic-pituitary-adrenal (HPA) axis (2—5). In the rat both histamine  $H_1$  and  $H_2$ -receptors are involved in stimulation of the HPA axis (4, 6).

Nitric oxide (NO) may act as a neuronal messenger and neuromodulator in the brain (7). NO plays an essential role in modulating many neuroendocrine responses (8). Nitric oxide synthase (NOS) is localized in hypothalamic neurones which regulate the activity of the HPA axis. NOS activity, immunoreactivity and NOS mRNA are present within the hypothalamic paraventricular nucleus (PVN), the main locus of corticotrophin releasing hormone (CRH) synthesis (9), and neuronal NOS is a major NOS isoform in rat hypothalamus (8, 10). NOS is colocalized with CRH and vasopressin secreting neurones in the hypothalamic paraventricular nucleus (11). Nitric oxide might play physiological role in the response of HPA axis to neuropeptides (12—14) and neurotransmitters stimuli (15). It may affect both hypothalamic CRH and pituitary ACTH release and, consequently, corticosterone secretion.

Sympathomimetic agonists enhance the basal release of NO and noradrenaline increases synthesis of NO *in vitro* in the incubated medial basal hypothalamus via  $\alpha_1$ -adrenergic receptors (16). NO mediates the stimulation of ACTH and corticosterone secretion induced by central activation of  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors (15). A possible involvement of NO in central histaminergic stimulation of the HPA axis *in vivo* is unknown.

Histamine, by mediation of  $H_1$ -receptor, activates phospholipase C which catalyses phospholipid breakdown to inositol-1,4,5 triphosphate ( $IP_3$ ) and diacylglycerol. In many tissues  $IP_3$  augments the intracellular  $Ca^{2+}$  concentration which, in turn, can activate NO synthase (17). The histamine  $H_1$ -receptor induced mobilization of intracellular  $Ca^{2+}$  can be the major regulatory factor for the production of NO.  $Ca^{2+}$  influx is required for stimulation by histamine of NO production in human endothelial cells (18).

The effects of the  $H_2$ -receptor are mediated primarily via adenylyl cyclase-dependent production of cAMP, but in some cells these effect are probably mediated by activation of the phosphoinositide signaling cascade through an independent G protein-dependent mechanism. Stimulation of histamine  $H_2$  receptors by dimaprit *in vitro* inhibited rat brain nNOS in a concentration dependent manner (19).

A possible involvement of NO in central histaminergic stimulation of the HPA axis *in vivo* is not known. The purpose of the present study was to investigate possible role of NO in central stimulation by histamine and histamine  $H_1$ - and  $H_2$ -receptor agonists of the HPA activity in conscious rats.

## MATERIAL AND METHODS

*Animals*

The experiment were carried out on male Wistar rats, weighing 180–230 g, which were kept 6–7 in groups for at least 1 week before use in a 12 h light-dark cycle. The rats were feed ad libitum on commercial food and tap water. For intracerebroventricular injections, the skulls of rats were prepared one day earlier under light ether anaesthesia (20). The rats remained in their home cages until they were scheduled for treatment. The experiments were performed in accordance with bioethical requirements.

*Experiments*

The rats were randomly assigned to one of the experimental groups (6 animal each). Control rats were injected with 10  $\mu$ l of saline or vehicle into the right cerebral ventricle; the experimental animals were injected with histaminergic agonists contained in 10  $\mu$ l of solvent: histamine, HTMT, a  $H_1$ -receptor agonist, and amthamine, a  $H_2$ -receptor agonist, or with 7-nitroindazole 15 min before each histaminergic agonist. 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. At first, dose-response studies were carried out to determine the most effective doses of HTMT and amthamine in inducing ACTH and corticosterone secretion. Rats were injected i.c.v. with different doses (1, 10, 20, 50, 75 and 100  $\mu$ g) of histamine receptor agonists. The most effective doses were 50  $\mu$ g for histamine, and 75  $\mu$ g for HTMT and amthamine.

*ACTH and corticosterone determinations*

Trunk blood samples were collected on ice in conical plastic tubes containing 200  $\mu$ l 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  $-20^\circ\text{C}$  until the time of assay. Plasma ACTH concentrations were measured using a double antibody  $^{125}\text{I}$  radioimmunoassay obtained from CIS Bio International, and were calculated as pg/ml of the plasma (21). The concentration of corticosterone was measured fluorometrically and expressed as  $\mu$ l/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 (Tocris Cookson, Bristol, UK) and 7-nitroindazole (Sigma). Histamine and amthamine were dissolved in sterile saline, HTMT was dissolved in concentrated ethanol and diluted with sterile water and 7-nitroindazole was suspended in 1% Tween. All the drug solutions were prepared immediately before use; the doses used are expressed in terms of salts.

*Statistics*

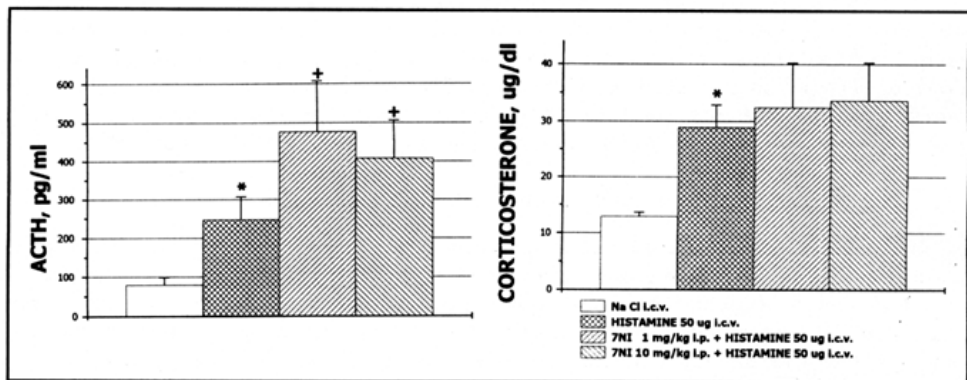
The results were calculated as a group mean  $\pm$  standard error of the mean. A statistical evaluation was performed by an analysis of variance, followed by individual comparisons with Duncan's test. The results were considered significantly different when  $p < 0.05$ .

## RESULTS

The vehicles used for dissolving HTMT and 7-nitroindazole, diluted ethanol and 1% Tween, respectively, given alone in respective volumes i.c.v. or i.p. 1h earlier did not substantially alter the basal plasma hormone levels. Likewise, 7-NI alone given i.p. in doses used in the present experiment (1 and 10 mg/kg) did not affect basal ACTH and corticosterone levels.

*Effect of 7-nitroindazole on histamine-induced ACTH and corticosterone secretion*

Histamine (50  $\mu$ g) administered i.c.v. induced a significant increase in plasma ACTH and corticosterone levels 1h after injection. 7-NI (1 and 10 mg/kg i.p.), a neuronal NOS inhibitor, given 15 min prior to histamine markedly augmented the histamine-induced ACTH response (*Fig. 1*). In a lower dose (1 mg/kg) 7-NI induced a more potent increase in ACTH secretion than given in a larger dose (10 mg/kg), from the control level of 246 pg/ml to 470 pg/ml and 408 pg/ml, respectively. The histamine-induced corticosterone response was not substantially altered by pretreatment with 7-Ni.



*Fig. 1.* Effect of 7-nitroindazole (7-NI) on the histamine-induced plasma ACTH and corticosterone levels. 7-NI was injected i.p. 15 min before i.c.v. histamine and 1 h after the last injection the rats were decapitated. In *Fig. 1–3* values represent the mean  $\pm$  SEM of 6 rats. \*  $p < 0.05$  vs. saline controls and +  $p < 0.05$  vs. histamine or histamine receptor agonist-treated group.

*Effect of 7-NI on HTMT-induced ACTH and corticosterone secretion*

HTMT (75  $\mu$ g i.c.v.), histamine  $H_1$ -receptor agonist, elicited a significant stimulation of ACTH and corticosterone secretion 1h after administration. A lower dose (50  $\mu$ g i.c.v.) was less effective and still lower doses (1–20  $\mu$ g i.c.v.) did not visibly alter the resting plasma ACTH and corticosterone levels. Pretreatment with 7-NI in both doses used (1 and 10 mg/kg i.p.) completely abolished the HTMT-elicited ACTH and corticosterone responses (*Fig. 2*). This results strongly suggest that endogenous neuronal NO participates considerably in central stimulation of the HPA activity.

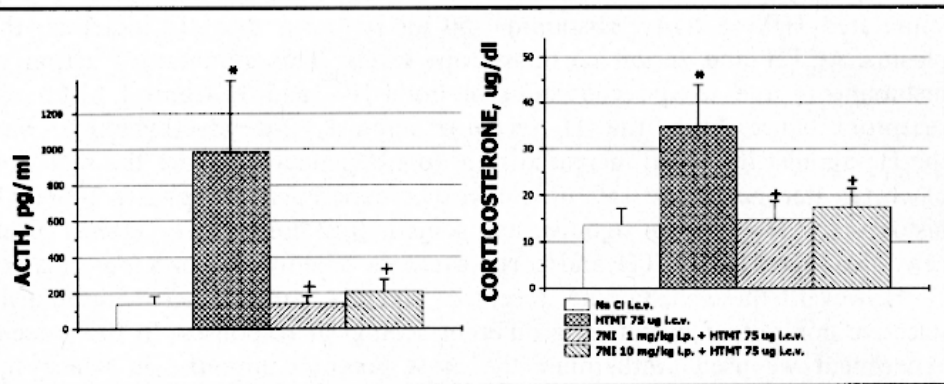


Fig. 2. Effect of 7-NI on the histamine-trifluoromethyl-toluidide (HTMT)-induced plasma ACTH and corticosterone levels. See legend to Fig. 1.

### Effect of 7-nitroindazole on amthamine-induced ACTH and corticosterone secretion

Amthamine, a highly selective standard histamine  $H_2$ -receptor agonist given i.c.v. to conscious rats induced a significant increase in ACTH and corticosterone secretion in a dose of 75  $\mu$ g. Lower doses were not markedly effective in this respect. Pretreatment with 7-NI slightly lowered the amthamine-induced ACTH response, and did not markedly affect the corticosterone response (Fig. 3).

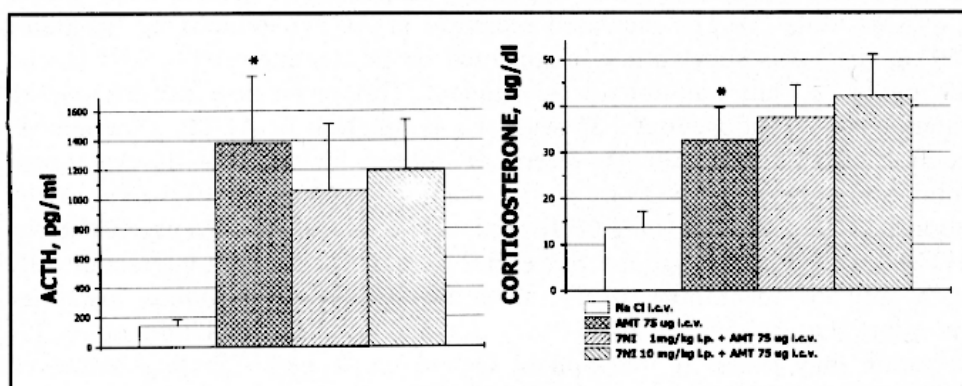


Fig. 3. Effect of 7-NI on the amthamine (AMT)-induced plasma ACTH and corticosterone levels. See legend to Fig. 1.

### DISCUSSION

In the present study we demonstrated that histamine, HTMT, a selective and potent  $H_1$ -receptor agonist, and amthamine, a specific  $H_2$ -receptor agonist, given into the lateral cerebral ventricle in conscious rats, considerably

stimulated HPA activity. Histamine (50  $\mu\text{g}$  i.c.v.) significantly increased the plasma ACTH and serum corticosterone levels. This stimulatory action of histamine is due to the activation of both  $\text{H}_1$ - and  $\text{H}_2$ -central histamine receptors, since both the  $\text{H}_1$ -receptor agonist, 2-pirydyethylamine, and the  $\text{H}_2$ -agonist dimaprit, increased in a dose-dependent manner the secretion of corticosterone in the rat, in our earlier experiment. Likewise, 2-methylhistamine, a  $\text{H}_1$ -receptor agonist, and 4-methylhistamine, a  $\text{H}_2$ -agonist given i.c.v. also increased ACTH and corticosterone secretion in conscious rats (4).

However, these histamine receptor agonists are neither satisfactorily selective nor potent in inducing different biological responses. In the present experiment we used amthamine, the most potent compound in which the imidazole ring of histamine was replaced by 2-aminothiazole ring. Amthamine combines a high  $\text{H}_2$ -receptor selectivity with a potency slightly higher compared with histamine, both in vitro and in vivo (22). Likewise, we used the histamine-trifluoromethyl-toluidide derivative (HTMT) which is highly selective  $\text{H}_1$ -receptor agonist in release of NO and the opening of ATP-sensitive  $\text{K}^+$  channels in vascular bed (23). Both HTMT and amthamine elicited a potent increase in ACTH and corticosterone secretion in the present experiment.

To test the effect of NO synthesis inhibition on central histaminergic stimulation of ACTH and corticosterone secretion, we used the brain-selective NO-synthase blocker, 7-nitroindazole, which selectively inhibits both rat and mouse brain NO-synthase with a potency similar to, or greater than, that of L-NAME (24, 25). The increased secretion of ACTH induced by histamine (50  $\mu\text{g}$  i.c.v.) was substantially augmented by pretreatment with 7-NI (1 and 10 mg/kg i.p.) but was not dose-dependent. This result does not exclude an interference of endogenous NO with the stimulation of ACTH secretion via central histamine  $\text{H}_1$ - or  $\text{H}_2$ -receptors. Indeed, in the present experiment inhibition of neuronal NOS by pretreatment with 7-NI (1 or 10 mg/kg) totally abolished the increase in ACTH and corticosterone secretion elicited by HTMT, a  $\text{H}_1$ -receptor agonist. Since endogenous NO does not interact directly with any of membrane neurotransmitter receptors, including histamine receptor, the interference of HTMT and NO in the stimulation of ACTH secretion may occur at the level of second messengers signaling pathways. Histamine, via  $\text{H}_1$ -receptor, is known to induce mobilization of intracellular  $\text{Ca}^{2+}$  and NO generation in endothelial cells and the opening of a  $\text{K}^+$ ATP channels (18, 23, 26). Therefore the inhibition of NO synthesis by 7-NI in neuronal structures involved in central stimulation of the histamine  $\text{H}_1$ -receptor-induced HPA response in our present experiment may be responsible for the total reduction of ACTH and corticosterone secretion stimulated by HTMT. This finding also suggests that endogenous NO is a strategic mediator of the HPA response to  $\text{H}_1$ -receptor stimulation.

Activation of histamine H<sub>1</sub>-receptor, besides NO induces also prostaglandins (PGs) generation. Prostaglandins in the brain are known to stimulate the HPA axis and mediate the stimulatory action of adrenergic signaling pathways (27). They also interact with histamine in the stimulatory regulation of ACTH secretion (28). However, it is unclear in which way PGs interact with endogenous NO in histaminergic regulation of the HPA axis activity.

The stimulatory effect of amthamine (75 µg i.c.v.), a selective and potent histamine H<sub>2</sub>-receptor agonist, on ACTH secretion was moderately diminished by pretreatment of rats with 7-NI (1 and 10 mg/kg i.p.). This diminution did not attain the level of statistical significance. This finding indicates that endogenous NO is not markedly involved in a potent stimulatory action of amthamine on ACTH and corticosterone secretion. Histamine H<sub>2</sub>-receptor is linked to the adenylyl cyclase-dependent production of cAMP. This intracellular signaling pathway mediates β-adrenergic receptor stimulated responses. Stimulation of ACTH and corticosterone secretion by i.c.v. isoprenaline, a non selective β-adrenergic receptor agonist, was only slightly diminished by L-NAME, a NOS inhibitor in our earlier study (15). This finding further suggests that endogenous NO is not significantly involved in the adenylate cyclase pathway mediated responses. This may explain the lack of inhibition by 7-NI of the amthamine-induced ACTH secretion in the present experiment.

Our present results show that endogenous NO is considerably involved in the stimulation of ACTH via histamine H<sub>1</sub>-receptors but not via H<sub>2</sub>-receptors. These two receptor subtypes are known to elicit opposing physiological responses in many tissues.

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