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J. BUGAJSKI, A. GĄDEK-MICHALSKA, R. GŁÓD, J. BORYCZ, *A. J. BUGAJSKI

BLOCKADE OF NITRIC OXIDE FORMATION IMPAIRS ADRENERGIC-INDUCED ACTH AND CORTICOSTERONE SECRETION

Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, Cracow
*Department of Pathophysiology, Jagiellonian University, School of Medicine, Cracow, Poland

It has been suggested that adrenergic agents might modulate the L-arginine-NO pathway. Sympathomimetic agonists enhance the basal release of NO, and noradrenaline increases the synthesis of nitric oxide synthase (NOS) in the medial basal hypothalamus in vitro. In the present study possible involvement of NO in central stimulation of the hypothalamic-pituitary-adrenal (HPA) axis by adrenergic agents was investigated in conscious rats. The nitric oxide synthase blocker N^{ω}-nitro-L-arginine methyl ester (L-NAME 2 and 10 μg) was administered intracerebroventricularly (icv) 15 min before the adrenergic agonist given by the same route; 1 h later the rats were decapitated. Plasma levels of ACTH and corticosterone were measured. L-NAME significantly diminished the ACTH and corticosterone response to phenylephrine (30 μg), an α_1-adrenergic receptor agonist. These hormone responses to clonidine (10 μg), an α_2-receptor agonist, were dose-dependently suppressed or totally abolished by L-NAME. A significant rise in the ACTH and corticosterone secretion induced by isoprenaline (10 μg), a β-adrenergic receptor agonist, was only moderately diminished by pretreatment with L-NAME. These results indicate that NOS is considerably involved in central stimulation of the HPA axis by α_1- and α_2-adrenergic receptor agonists, and that NO mediates the stimulatory action of these agonists on ACTH and corticosterone secretion. The stimulation induced by β-adrenergic receptors is only moderately affected by endogenous NO.

Key words: *central adrenergic receptors, ACTH, corticosterone, NOS blocker, NO.*

INTRODUCTION

Diffusible messenger molecule nitric oxide is produced by nitric oxide synthase (NOS) isoforms by oxidation of one of the guanidino nitrogens of L-arginine. NO functions as an important physiological mediator in synaptic and non-synaptic communication in the central nervous system (1). Constitutive nitric oxide synthase is expressed in the central and peripheral nervous

system and is known as brain or neuronal NOS. A nitric oxide synthase-like activity, NOS immunoreactivity and NOS mRNA are present within the hypothalamic paraventricular nucleus (PVN), and nNOS is a major NOS isoform in rat hypothalamus (2, 3). NOS is also present in both the posterior and anterior pituitary gland and in the hypophyseal portal vasculature which delivers hypothalamic releasing factors to the anterior pituitary. NO modulates the secretion of corticotropin releasing hormone (CRH) (4, 5) from rat hypothalamic explants or mediobasal hypothalami *in vitro* and it considerably affects the CRH- and vasopressin-induced secretion of ACTH and corticosterone *in vivo* (6, 7). The endogenously formed NO has been reported to facilitate noradrenaline release from rat hippocampal slices (8—10), or to inhibit the release of noradrenaline and dopamine from the medial basal hypothalamus of the rat (11). It has also been suggested that adrenergic agents modulate the L-arginine-NO pathway. It is well known that acute *in vitro* exposure of the endothelium to sympathomimetic agonists enhances the basal release of NO. Noradrenaline was reported to increase the synthesis of NO *in vitro* in the incubated medial basal hypothalamus *via* α_1-adrenergic receptors (12). Chronic treatment with noradrenaline or isoprenaline also enhanced pineal NOS activity in rats (13). The exact role of endogenous NO in stimulation of the HPA axis by adrenergic receptor agonists in conscious rats is still unknown.

The purpose of the present study was to determine the role of NO in stimulation of the HPA axis by activation of central α_1-, α_2- and β-adrenergic receptors in conscious rats. Neuronal NOS was blocked by its antagonist, L-NAME. Since peripheral administration of L-NAME gives little selectivity of inhibition between eNOS and nNOS, this compound was administered directly into the CNS to increase the specifity for nNOS (14).

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 (icv) injections, the skulls of rats were prepared one day earlier under light ether anesthesia.

Experiments

The rats were randomly assigned to one of the experimental groups (6 animals each). Control rats were injected with 10 µl of saline into the right cerebral ventricle; the experimental animals were injected with adrenergic agonists contained in 10 µl of saline: phenylephrine (30 µg),

clonidine (10 μg) and isoprenaline (20 μg), or with L-NAME (2 and 10 μg) 15 min before each adrenergic agonist.

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

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 conical plastic tubes containing 200 μl of a solution of EDTA, 5 mg/ml, and aprotinin, 500 TIU (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 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.

Drugs

The following drugs were used: L-phenylephrine hydrochloride, DL-isoproterenol hydrochloride, Nω-nitro-L-arginine methyl ester (L-NAME) (Sigma) and clonidine (Boehringer). Drugs for icv administration 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. 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

Effect of L-NAME on the phenylephrine-induced ACTH and corticosterone secretion

Phenylephrine (30 μg), an α_1-adrenergic receptor agonist, administered icv, significantly raised plasma ACTH and corticosterone levels 1 h after injection. The nitric oxide synthase blocker L-NAME, given alone in doses administered to control rats in the present experiment, had no marked influence on basal plasma ACTH and corticosterone levels (15). L-NAME (2 and 10 μg), given icv 15 min prior to phenylephrine, considerably diminished, by 69 and 82%, respectively, the phenylephrine-induced plasma ACTH concentration. The

corticosterone response was also significantly diminished, by 70 and 56%, respectively, by the above doses of L-NAME (*Fig. 1*).

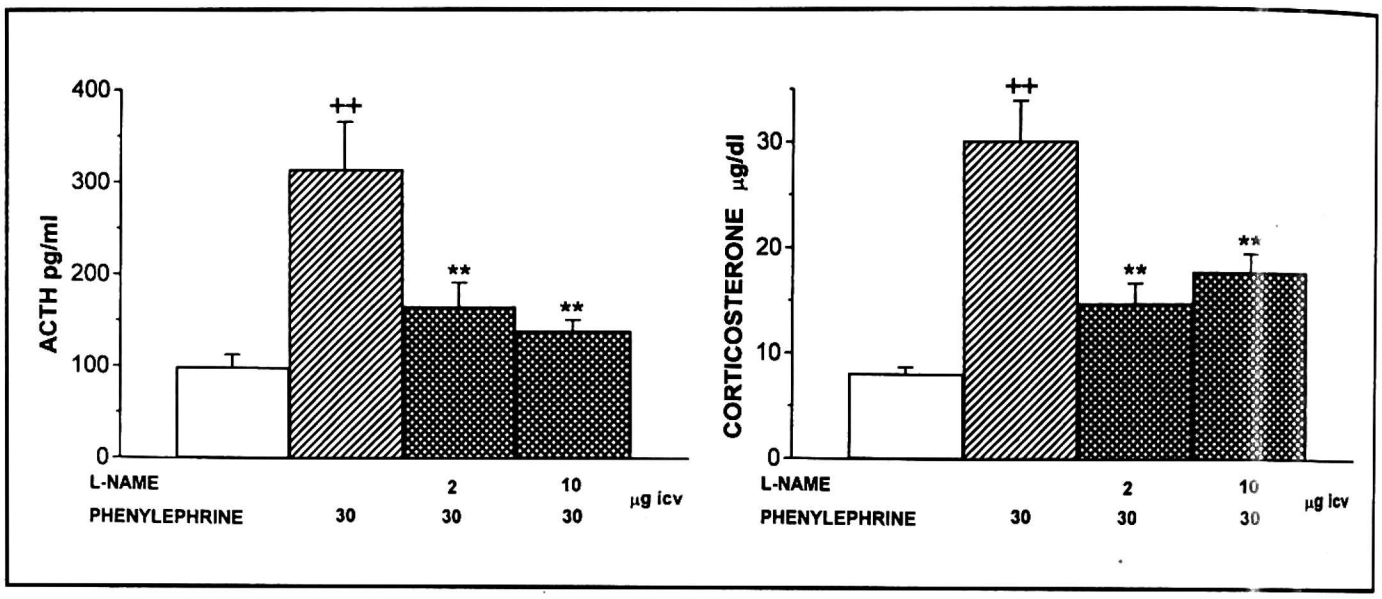

Fig. 1. Effect of L-NAME on phenylephrine-induced plasma ACTH and corticosterone levels. Both agents were administered icv, L-NAME 15 min before phenylephrine. 1 h after the last injection the rats were decapitated. In *Fig. 1—3* values represent the mean ± SEM of 6 rats. $^{+}p < 0.05$ and $^{++}p < 0.01$ vs. saline controls, $*p < 0.05$ and $**p < 0.01$ vs. adrenergic agonist-treated group.

Effect of L-NAME on the clonidine-induced ACTH and corticosterone secretion

A significant increase in ACTH secretion, elicited by clonidine (10 µg icv), an α_2-adrenergic receptor agonist, was either considerably diminished or totally abolished by pretreatment with L-NAME (2 and 10 µg icv) (*Fig. 2*). The clonidine-induced corticosterone response was also dose-dependently lowered, by 43 and 62%, respectively, by icv pretreatment with L-NAME in the two doses used (*Fig. 2*).

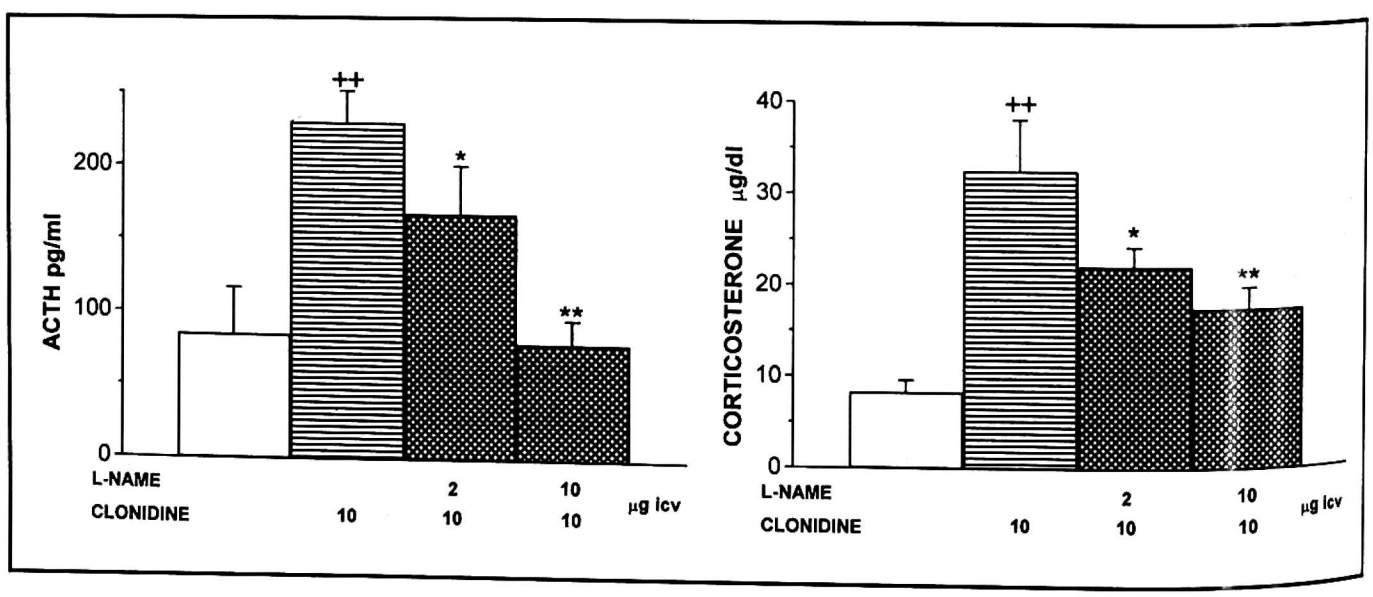

Fig. 2. Effect of L-NAME on clonidine-induced plasma ACTH and corticosterone levels. L-NAME was given 15 min before clonidine. See legend to *Fig. 1.*

Effect of L-NAME on the isoprenaline-induced ACTH and corticosterone response

Isoprenaline (20 μg icv), a β-adrenergic receptor agonist, significantly raised both ACTH and corticosterone secretion 1 h after administration. Pretreatment with L-NAME (2 and 10 μg icv) markedly diminished the isoprenaline-induced rise in ACTH secretion, by 30 and 24%, respectively, and it lowered corticosterone secretion to a similar extent, by 30 and 36%, respectively, (*Fig. 3*). However, those alterations were not statistically significant (*Fig. 3*).

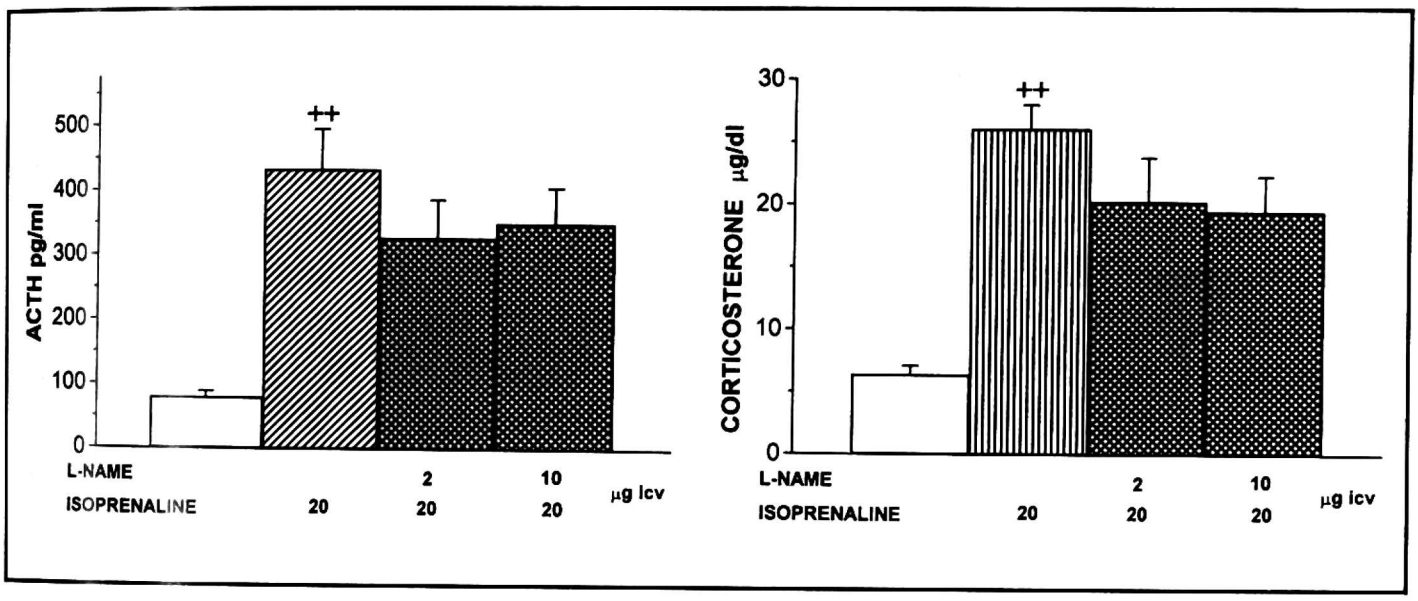

Fig. 3. Effect of L-NAME on isoprenaline-induced plasma ACTH and corticosterone levels. L-NAME was given 15 min before isoprenaline. See legend to *Fig. 1.*

DISCUSSION

In the present experiment the hypothalamic-pituitary-adrenal axis was activated by α_1- α_2- and β-adrenergic receptor agonists given icv to conscious rats. After icv administration, these agonists stimulate mainly the corticotropin-releasing hormone containing neurons in the hypothalamic paraventricular nucleus (16—18). CRH neurons project to the median eminence, and the CRH released from these neurons stimulates the secretion of ACTH from anterior pituitary corticotrops; in turn ACTH, regulates the release of corticosterone from the adrenal cortex.

Both hypothalamic and anterior pituitary α_1-adrenergic receptors are known to participate in activation of the HPA axis in intact animals (16—19). The main targets of icv phenylephrine action are α_1-adrenoceptors on CRH-secreting neurons in the hypothalamic PVN. Any substantial activation of α_1-adrenoceptors on anterior pituitary corticotrops by icv phenylephrine which would stimulate ACTH secretion seems unlikely since in our earlier experiment prazosin, an α_1-adrenergic receptor blocker which does not easily penetrate the blood-brain barrier, given ip was unable to markedly impair the icv phenylephrine-induced corticosterone response (20).

We found that L-NAME (2 and 10 μg), a NOS inhibitor, given icv considerably reduced the increase in ACTH and corticosterone secretion induced by subsequent icv administration of phenylephrine (30 μg), an α_1-adrenergic receptor agonist. Considerable inhibition of the phenylephrine-induced ACTH and corticosterone responses by L-NAME indicates that endogenous NO significantly mediates these responses. In the present experiment the extent of L-NAME-evoked inhibition of the phenylephrine-elicited HPA response was equal to a similar inhibition induced by prazosin, an α_1-adrenergic receptor antagonist (21). The above findings indicate that NO is considerably involved in central stimulation of the HPA axis *via* α_1-adrenergic receptors. Likewise, the phenylephrine-evoked α_1-adrenergic stimulation of cultured neonatal cardiac myocytes significantly enhanced the NO production stimulated by IL-1β, the effect of phenylephrine being completely abolished in the presence of L-NMMA (22). Phenylephrine exerts its effect *via* α_1-adrenergic receptors which stimulate the release of NO from nitricoxidergic neurons in the hypothalamic PVN. Nitric oxide activates guanylate cyclase and induces the release of CRH into hypophyseal portal vessels. In the present experiment the NOS antagonist considerably blocked that action of phenylephrine.

Clonidine (10 μg icv), an α_2-adrenergic agonist, significantly enhanced both ACTH and corticosterone secretion 1 h after its administration. The NOS antagonist L-NAME (2 and 10 μg icv) either considerably reduced or totally abolished the clonidine-induced ACTH response and it significantly lowered the corticosterone response, by 43 and 62%, respectively. According to our earlier findings, clonidine stimulates centrally and to the same extent the HPA axis *via* presynaptic α_2- and postsynaptic α-adrenergic receptors (21, 23). To date, it is not known to what extent the NOS inhibitor used in the present study interferes with the clonidine-stimulated HPA response at both these receptor sites. The endogenous NO diffuses easily in the neuronal tissue and may interfere with signal transduction at pre- and postsynaptic sites. Therefore at least part of the inhibitory action of L-NAME on the clonidine-induced HPA response may be attributed to postsynaptic α-adrenoceptors. Stimulation of prejunctional α_2-adrenoceptors can inhibit NO release from non-adrenergic-non-cholinergic nerves in the canine ileocolonic junction (24); however, it is not known whether icv clonidine, acting *via* α_2-presynaptic receptors, may evoke a similar effect in the hypothalamic neurons involved in activation of CRH release. Stimulation by clonidine of ACTH secretion and considerable inhibition of this secretion by the NOS antagonist L-NAME make such an assumption unlikely. Clonidine decreases cyclic guanosine 3',5'-monophosphate which is known to be potently activated by NO. Whether and in what way the antagonism between the action of clonidine and NO may be responsible for stimulation or inhibition of the CRH/ACTH response, remains to be further elucidated.

Isoprenaline (20 μg), a non selective β-adrenergic receptor agonist, given icv evoked a significant rise in plasma ACTH and corticosterone levels. NOS

inhibitor L-NAME only moderately and insignificantly diminished, by ca 30% at the maximum, the isoprenaline-induced ACTH and corticosterone response. These findings indicate that endogenous NO is not significantly involved in the HPA response to β-adrenergic receptor stimulation, which is in contrast to a major role of NO in mediating α_1- and α_2-adrenergic stimulation of the HPA axis found in the present study. We also observed similar weak involvement of NO in the adrenaline-induced stimulation of the HPA axis *via* β-adrenergic receptors (data not shown). On the other hand, nitric oxide was reported to inhibit the isoprenaline-induced inotropic effects in rat heart (25). Similarly, β-adrenergic receptors mediated responses in isolated hearts and cardiomyocytes were attenuated by both NO and guanylate cyclase inhibitors (26). Moderate diminution of the isoprenaline-induced HPA response by L-NAME may be related to relatively weak sensitivity of the cAMP signalling system to modulation by endogenous nitric oxide.

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Autor's address: J. Bugajski, Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12 Str., 31-343 Kraków, Poland.
E-mail: bugajski@if-pan.krakow.pl