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

INFLUENCE OF NITRIC OXIDE SYNTHASE INHIBITORS ON THE VASOPRESSIN-INDUCED PITUITARY-ADRENOCORTICAL ACTIVITY AND HYPOTHALAMIC CATECHOLAMINE LEVELS

Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

In the present study the role of endogenous nitric oxide (NO) in the vasopressin-induced ACTH and corticosterone secretion was investigated in conscious rats. Vasopressin (AVP 5 μg/kg ip) considerably augmented ACTH and corticosterone secretion. L-arginine (120 and 300 mg/kg ip) did not significantly alter the AVP-induced secretion of those hormones. Nitric oxide synthase (NOS) blockers N^{ω}-nitro-L-arginine (L-NNA) and its methyl ester (L-NAME) given ip 15 min before AVP markedly increased the AVP-induced ACTH secretion. L-NNA (2 mg/kg) more potently and significantly increased the AVP-induced ACTH secretion, whereas L-NAME elicited a weaker and not significant effect. Both those NOS antagonists intensified significantly and to a similar extent the AVP-induced corticosterone secretion. L-arginine (120 mg/kg ip) reversed the L-NNA-induced rise in the AVP-stimulated ACTH secretion and substantially diminished the accompanying corticosterone secretion. Neither vasopressin alone nor in combination with L-arginine and L-NAME evoked any significant alterations in the hypothalamic noradrenaline and dopamine levels. L-NNA (2 and 10 mg/kg ip) elicited a dose dependent and significant decrease in the hypothalamic noradrenaline level. The hypothalamic dopamine level was not significantly altered by any treatment. These results indicate that in conscious rats endogenous NO has an inhibitory influence on the AVP-induced increase in ACTH and corticosterone secretion. L-NNA is significantly more potent than L-NAME in increasing the AVP-induced ACTH secretion. This may be connected with a considerable increase by L-NNA of hypothalamic noradrenergic system activation which stimulates the pituitary-adrenal axis in addition to specific inhibition of NOS.

Key words: *vasopressin, ACTH, corticosterone, nitric oxide, NOS blockers, hypothalamic catecholamines.*

INTRODUCTION

Recent data indicates that nitric oxide (NO) may act as a neuronal messenger and neuromodulator in the brain (1). In the central nervous system it is synthesized by the Ca^{2+}-calmodulin-dependent enzyme, NO-synthase,

from L-arginine as a substrate. In neurons the NOS enzyme is present under physiological conditions. NO participates in several physiological functions in the central nervous system. NOS is widely distributed in several brain areas (2) involved in the synthesis and storage of vasopressin, particularly in the hypothalamic supraoptic and paraventricular nuclei (3) and in nerve terminals of the posterior pituitary. NOS is colocalized with magnocellular oxytocin and arginine vasopressin (4). Nitric oxide directly and specifically inhibits the stimulated release of AVP from rat hypothalamic explants and from the supraoptic nucleus *in vitro* (5, 6), whereas it can centrally stimulate vasopressin release in conscious rats (7). On the other hand, NO can mediate various actions of AVP in peripheral tissues e.g. cytokine stimulation of rat cardiac myocytes and renal vasodilation (8—10). Nitric oxide is involved in modulation of various neuroendocrine responses such as secretion of CRH and ACTH (11—13). NO may also act on the neurotransmitters involved in the control of the HPA axis activity, such as catecholamines (14—16). Central catecholamines from axons projecting to the PVN activate α_1-adrenergic receptors that selectively stimulate the release of both CRH and AVP in the portal capillary plexus (17). Noradrenaline activates, *via* α_1-adrenergic receptors, AVP-containing neurons also in the hypothalamic supraoptic nucleus (18). Nitric oxide can inhibit corticosterone secretion elicited by vasopressin given both systemically and intracerebroventricularly (19). Nevertheless, the role played by NO and the site of interaction with the AVP-induced HPA stimulation whether central, pituitary or/and adrenal gland, remain unclear. Likewise, adrenergic component in this interaction has not been determined.

This study examines the effect of NOS blockers and the NO substrate on the vasopressin-induced ACTH and corticosterone secretion, as well as on hypothalamic catecholamine levels in conscious rats.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 200—230 g were housed in groups of 7 at a room temperature of $20 \pm 2°C$ and on a daylight cycle at last one week before the experiment. The animals had free acces to standard rodent laboratory food and tap water.

Experiments

The rats were randomly assigned to one of the experimental groups, 6 animals each. They were injected ip with saline or the required drug in a volume of 1 ml/kg. In the control group the rats received ip injection of saline, the experimental groups were injected with vasopressin 5 μg/kg, or L-arginine 120 or 300 mg/kg 15 min before vasopressin; with L-NAME 2 mg/kg or

L-NNA 2 mg/kg 15 min before vasopressin and the last group received L-arginine + L-NNA before vasopressin.

In order to avoid interference with the circadian rhythm in ACTH and corticosterone levels, all experiments were performed between 9 and 10 a.m. and all decapitations were carried out between 10 and 11 a.m. when plasma hormone concentrations are low in the 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 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 were calculated as pg/ml of plasma. The concentration of corticosterone was measured fluorometrically and expressed as µg per 100 ml.

Hypothalamic dopamine and noradrenaline determinations

For dopamine and noradrenaline determinations, the rats were decapitated at the required time, their brains were quickly removed, placed on glass plates kept on ice and washed with an ice-cold saline. The cerebella were discarded and the hypothalami were isolated and stored at −80°C until further use. For an HPLC assay frozen hypothalami were put into approx. 10 vol. of ice-cold 0.1 M $HClO_4$ containing 5 mM of ascorbic acid and 25 µg/l of 3, 4 dihydrobenzylamine (internal standard); then they were weighed and homogenized with an Ultra-Turrax homogenizer (10 s at 20000 rpm). The homogenates were centrifuged at 14000 × g and the supernatants were subsequently filtered out through 0.22 µm RC-58 membranes (BAS MF-1 centrifugal microfilters). The filtrates were injected into the HPLC system. A BAS-400 liquid chromatograph (BAS, USA), equipped with an LC4B/17AT electrochemical detecter and 3 µg C_{18} Phase 2 analytical column (100 mm × 3 mm), coupled to a 7 µm C_{18} guard column (15 mm × 3 mm) was used. The mobile phase (36 mM citrate-28 mM phosphate buffer pH 3.5, containing 0.77 mM of EDTA and 5% methanol) was pumped at 0.9 ml/min through the column thermostatted at 32°C. The separated sample components of dopamine and noradrenaline were detected at an oxidation potential of 0.8 V. All the reagents were of analytical grade (Merck, Germany and Sigma, USA).

Statistical analysis

The data are presented as mean ± SEM. Statistical significance of differences between groups was estimated by an analysis of variance, followed by individual comparisons with the Duncan test. The results were considered significantly different if $p < 0.05$.

Drugs

[Arg^8]-vasopressin acetate salt, L-arginine hydrochloride, Nω-nitro-L-arginine and Nω-nitro-L-arginine methyl ester hydrochloride were purchased from Sigma. All drugs used in this study were dissolved in saline immediately before injection.

RESULTS

Effect of L-arginine on the vasopressin-stimulated ACTH and corticosterone secretion

Vasopressin (5 μg/kg ip) significantly increased the plasma ACTH level, from 20±4.4 to 218±69 pg/ml, measured 1 h after AVP injection. Pretreatment with L-arginine (120 mg/kg ip 15 min before AVP), a substrate for nitric oxide synthase, did not alter the AVP-induced plasma ACTH level. Used in a higher dose of 300 mg/kg ip, L-arginine diminished to 91±39 pg/ml, not significantly, the AVP-evoked plasma ACTH level (*Fig. 1*). Likewise, the AVP-stimulated significant increase in the serum corticosterone level, from 10±3.0 to 36±3.8 μg/dl, was not substantially altered by pretreatment with L-arginine in both the doses used (*Fig. 1*).

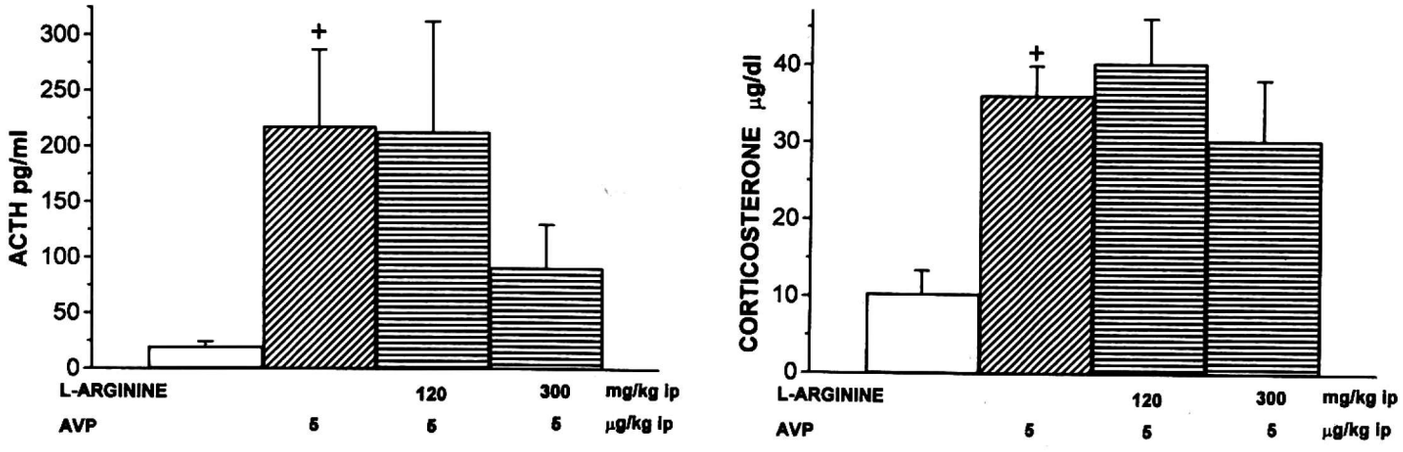

Fig. 1. Effect of L-arginine on the vasopressin (AVP)-induced ACTH and corticosterone secretion. L-arginine was injected ip 15 min before AVP. In *Fig. 1—4* one hour after the last injection the rats were decapitated. Values represent the mean±SEM of 6 rats. $^{+}p < 0.05$ and $^{++}p < 0.01$ vs. saline control group. $*p < 0.05$ and $**p < 0.01$ vs. AVP-treated group.

Effect of endogenous NO synthase blockers on the vasopressin-stimulated ACTH and corticosterone secretion

L-NAME (2 mg/kg ip) given 15 min prior to AVP substantially, but not significantly, increased the AVP-induced plasma ACTH level, from 255±57 to 364±96 pg/ml. In a higher dose (10 mg/kg ip), L-NAME did not elicit any further increase in the plasma ACTH level (data not shown). However, that NOS blocker significantly augmented the AVP-evoked rise in the serum corticosterone level, from 29±2.0 to 43±8.0 μg/dl (*Fig. 2*).

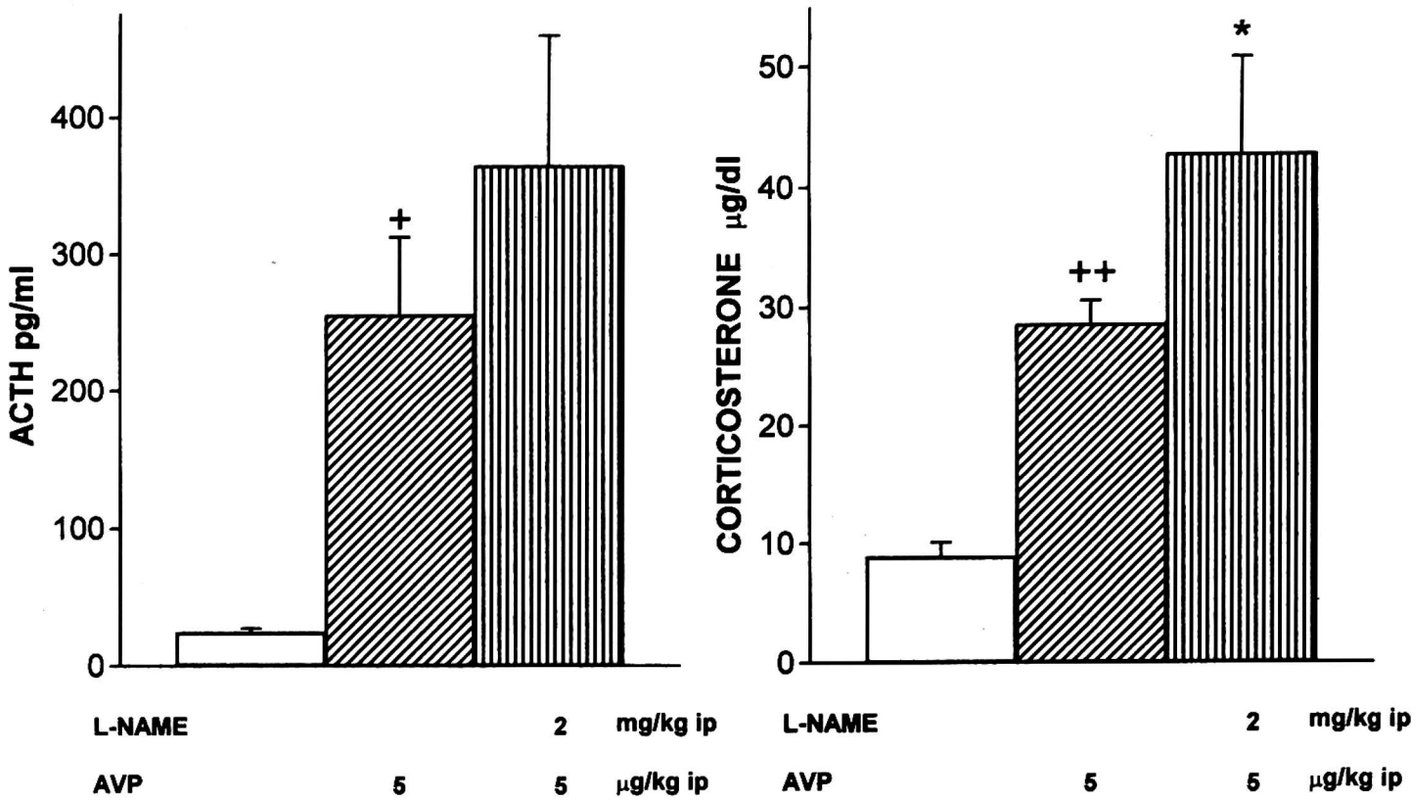

Fig. 2. Effect of L-NAME on AVP-induced plasma ACTH and corticosterone levels. L-NAME was injected ip 15 min before AVP. See legend to *Fig. 1.*

L-NNA (2 mg/kg ip), given 15 min before AVP, significantly and more potently than L-NAME augmented the AVP-stimulated increase in the plasma ACTH level, from 261 ± 57 to 468 ± 59 pg/ml. Likewise, that blocker also

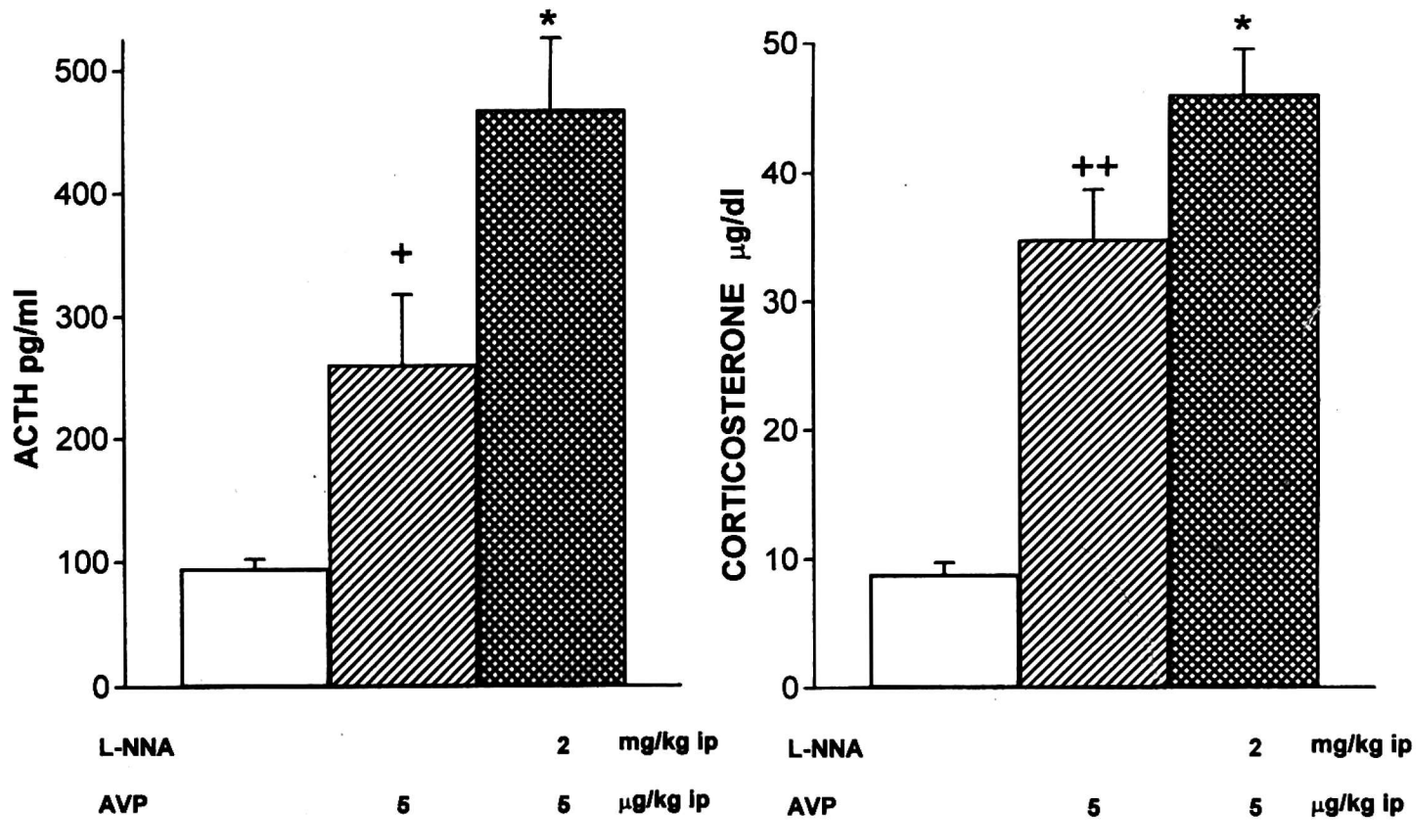

Fig. 3. Effect of L-NNA on AVP-induced plasma ACTH and corticosterone levels. L-NNA was injected ip 15 min before AVP. See legend to *Fig. 1.*

significantly increased the AVP-elicited rise in the serum corticosterone level, from 35±3.9 to 46±3.6 μg/dl (*Fig. 3*). These results show a more potent blocking activity of L-NNA than of L-NAME on endogenous NOS in the pituitary-adrenocortical axis.

Effect of L-arginine and L-NNA on the vasopressin-stimulated ACTH and corticosterone secretion

L-arginine (120 mg/kg ip) in a dose which by itself did not alter the basal plasma ACTH and corticosterone levels, given together with L-NNA (2 mg/kg ip) 15 min prior to AVP, reversed the AVP-elicited increase in the plasma ACTH level, from 525±66 to 244±70 pg/ml, i.e. below the AVP-evoked level (330±87 pg/ml). L-arginine also markedly, but not significantly, diminished the L-NNA-induced increase in the serum corticosterone level stimulated by AVP, from 52±2.9 to 43±2.4 (*Fig. 4*).

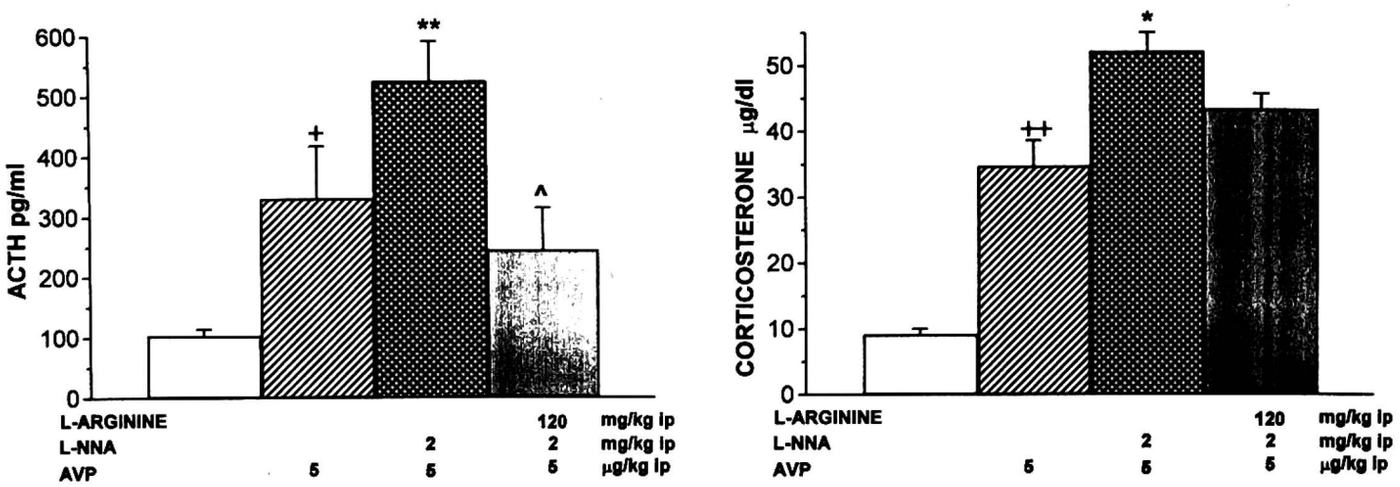

Fig. 4. Effect of L-arginine and L-NNA on AVP-induced plasma ACTH and corticosterone levels. L-arginine and L-NNA separately or together, were given 15 min before CRH. ^$p < 0.05$ vs. AVP+L-NNA treated group. See legend to *Fig. 1.*

Effect of L-arginine and NOS blockers on the vasopressin-induced hypothalamic catecholamine levels

Vasopressin (5 μg/kg ip) did not induce any significant alterations in the hypothalamic noradrenaline and dopamine levels 1 h after administration. L-arginine (120 mg/kg ip) and L-NAME (2, 5, 10 mg/kg ip) which by itself did not markedly affect the resting plasma ACTH and corticosterone levels (20) had no effect either on the AVP-induced hypothalamic noradrenaline or dopamine levels. Only minor alterations in the noradrenaline levels, which did not exceed 10% of the AVP-induced level and moderate changes in the dopamine levels were neither regular nor significant (*Table 1*). By contrast, L-NNA (2 and 10 mg/kg ip) considerably and dose-dependently diminished the AVP-induced hypothalamic noradrenaline levels, from 2714±153 to

1973±126 and 1636±93, respectively, leaving dopamine levels unaffected. Although both L-NAME and L-NNA increased the AVP-induced ACTH and corticosterone levels, they affected differently the hypothalamic noradrenaline levels.

Table 1. Effect of L-arginine and NOS blockers on the vasopressin-induced hypothalamic noradrenaline and dopamine levels.

TREATMENT	NORADRENALINE ng/g wet weight	DOPAMINE ng/g wet weight
Saline control	2443±286	368±45
$AVP_{5\mu g/kg}$	2466±150	495±107
L-$arginine_{120mg/kg}$+$AVP_{5\mu g/kg}$	2419±200	753±167
L-$arginine_{300mg/kg}$+$AVP_{5\mu g/kg}$	2572±148	593±101
Saline control	2545±214	566±157
$AVP_{5\mu g/kg}$	2525±163	777±185
L-$NAME_{2mg/kg}$+$AVP_{5\mu g/kg}$	2791±182	906±248
L-$NAME_{10mg/kg}$+$AVP_{5\mu g/kg}$	2264±157	527±68.9
Saline control	2712±195	633±142
$AVP_{5\mu g/kg}$	2714±153	767±148
L-$NNA_{2mg/kg}$+$AVP_{5\mu g/kg}$	1973±126**	676±204
L-$NNA_{10mg/kg}$+$AVP_{5\mu g/kg}$	1636±93**	650±91.8

L-arginine, L-NAME and L-NNA were injected ip 15 min before CRH. 1 h after the last injection the rats were decapitated, the brains were rapidly removed, hypothalami isolated on plates kept on ice and frozen at −70°C. Values represent the mean±SEM of 6 rats. **$p < 0.01$ vs. AVP-treated group.

DISCUSSION

Peripheral administration of L-arginine (120 mg/kg ip) or L-NAME alone did not significantly alter the basal ACTH and corticosterone levels (20), indicating that NO is not active under such circumstances. This result was in line with some earlier findings (13). The present experiment shows that L-arginine, a substrate for NOS, diminished more effectively the AVP-induced ACTH than corticosterone secretion, though these diminutions were statistically insignificant. Conversely, injection of arginine derivatives that interfere with the activity of NOS markedly augmented the stimulatory action of vasopressin on ACTH and corticosterone secretion. L-NNA more potently, by 79%, and significantly increased the AVP-induced ACTH secretion, whereas L-NAME elicited a lower (43%) and not significant increase. In our present

experiment (20) L-NAME was also less effective in potentiating the CRH-evoked ACTH response than the corticosterone secretion . This may suggest a more potent involvement of endogenous NO in steroidogenesis in the adrenal cortex than in the secretion of ACTH from the anterior pituitary. Although L-NNA and L-NAME have similar *in vitro* inhibitory potencies against neuronal NOS, their potencies *in vivo* may be different. L-NAME is a weak inhibitor of nNOS and has to be deestrified to L-NNA to inhibit nNOS. Such deestrification in the plasma is relatively slow, with a half-life of approximately 4 hr. In our experiment the blood was collected 75 min after systemic administration, when only part of the injected L-NAME was in an active deestrified form. Consequently, L-NAME may block the NO synthesis in a more prolonged way in the pituitary or adrenal gland, since N-NAME is rapidly deestrified in the brain (21) and L-NNA is also known to rapidly inactivate the neuronal NOS (22). NOS immunoreactivity and NOS mRNA are present within the PVN and SON of the hypothalamus (2, 3) and NOS is also present in a subpopulation of AVP-expressing CRH neurons. However, alterations in the NO synthesis in these central structures by ip injection of NOS blockers do not seem to represent any marked component of the L-NNA and L-NAME-induced augmentation of the AVP-stimulated ACTH and corticosterone secretion. Moreover, vasopressin given systemically activates directly its receptors on anterior pituitary corticotrops to stimulate ACTH secretion. Penetration of AVP to the hypothalamic structures involved in activation of the HPA axis is rather limited and seems to be of minor importance for the AVP-induced final ACTH secretory effects. The L-NNA-induced increase in the AVP-elicited ACTH response was completely reversed by L-arginine (120 mg/kg ip) injected together with L-NNA. This observation suggests a specific role of NO in the L-NNA-induced rise in the plasma ACTH level.

However, it is also possible that part of the NOS blockers-induced alterations in the vasopressin stimulated ACTH secretion results from their interaction with the neurotransmitters involved in the AVP-elicited pituitary-adrenal response. We observed a marked involvement of β-adrenergic receptors in the AVP-induced corticosterone secretion (23). In the present experiment neither vasopressin nor L-arginine or L-NAME itself or combined elicited any significant alterations in the hypothalamic noradrenaline and dopamine levels. Only L-NNA (2 and 10 mg/kg) significantly and dose-dependently decreased the AVP-induced hypothalamic noradrenaline level, which was accompanied with a potent increase in ACTH secretion. This decline in the hypothalamic noradrenaline level may suggest a considerably increased activation of the hypothalamic adrenergic system which is known to stimulate the activity of the HPA axis (17). Thus the present results suggest that L-NNA may potentiate the AVP-induced ACTH secretion by specific inhibition of NOS and stimulation of the hypothalamic noradrenergic system.

Conversely, L-NAME did not substantially affect the hypothalamic noradrenergic system when the HPA axis was stimulated with either AVP or CRH (20).

Both L-NAME and L-NNA induced a similar and significant increase in corticosterone secretion. The discrepancy between a greater increase by L-NAME in corticosterone than in ACTH secretion is not clear. It is possible that the increased central adrenergic activity induced by L-NNA influences ACTH but not corticosterone secretion. Moreover, vasopressin can directly affect adrenal steroid secretion as a potent paracrine stimulator (24—26). While vasopressin stimulates anterior pituitary corticotrops *via* V_{1b} receptors (27), it can activate adrenals *via* V_{1a} receptors (28). A possibility of a different interaction of NO with stimulation of the HPA axis via different vasopressin receptors is not known at present. Vasopressin and NOS blockers given systemically may directly affect the adrenal cortex and steroid secretion. This peripheral component of the interaction of NOS blockers with vasopressin stimulated corticosterone secretion awaits further elucidation.

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REFERENCES

1. Szabó C. Physiological and pathophysiological roles of nitric oxide in the central nervous system. *Brain Res Bull* 1996; 41: 131—141.
2. Vincent SR, Kimura H. Histochemical mapping of nitric oxide synthase in the rat brain *Neuroscience* 1992; 46: 755—784.
3. Calza L, Giardino L, Ceccatelli S. NOS mRNA in the paraventricular nucleus of young and old rats after immobilization stress. *Neuroreport* 1993; 4: 627—630.
4. Torres G, Lee S, Rivier C. Ontogeny of the rat hypothalamic nitric oxide synthase and colocalization with neuropeptides. *Mol Cell Neurosci* 1993; 4: 155—163.
5. Yasin S, Costa A, Trainer P, Windle R, Forsling ML, Grossman A. Nitric oxide modulates the release of vasopressin from rat hypothalamic explants. *Endocrinology* 1993; 133: 1466—1469.
6. Liu Q-S, Jia Y-S, Ju G. Nitric oxide inhibits neuronal activity in the supraoptic nucleus of the rat hypothalamic slices. *Brain Res Bull* 1997; 43: 121—125.
7. Ota M, Crofton JT, Festavan GT, Share L. Evidence that nitric oxide can act centrally to stimulate vasopressin release. *Neuroendocrinology* 1993; 57: 955—959.
8. Yamanoto K, Ikeda U, Okada K *et al.* Arginine vasopressin increases nitric oxide synthesis in cytokine-stimulated rat cardiac myocytes. *Hypertension* 1997; 30: 1112—1120.
9. Hirata Y, Haykawa H, Kakoki M *et al.* Receptor subtype for vasopressin-induced release of nitric oxide from rat kidney. *Hypertension* 1997; 29: 58—64.
10. Aki Y, Tamaki T, Kiyomoto H *et al.* Nitric oxide may participate in V_2 vasopressin-receptor-mediated renal vasodilation. *J Cardiovasc Pharmacol* 1994; 23: 331—336.
11. Costa A, Trainer P, Besser M, Grossman A. Nitric oxide modulates the release of corticotropin-releasing hormone for the rat hypothalamus in vitro. *Brain Res* 1993; 605: 187—192.
12. Karanth S, Lyson K, McCann SM. Role of nitric oxide in interlaukin 2-induced corticotropin-releasing factor release from incubated hypothalami. *Proc Natl Acad Sci USA* 1993; 90: 3383—3387.

13. Rivier C, Shen G. In the rat, endogenous nitric oxide modulates the response of the hypothalamic-pituitary-adrenal axis to interleukin-1β, vasopressin, and oxytocin. *J Neurosci* 1994; 14: 1985—1993.
14. Lonart G, Wang J, Johnson KM. Nitric oxide induces neurotransmitter release from hippocampal slices. *Eur J Pharmacol* 1992; 220: 271—272.
15. Satoh S, Murayama T, Nomura Y. Sodium nitroprusside stimulates noradrenaline release from rat hippocampal slices in the presence of dithiothreitol. *Brain Res* 1996; 733: 167—174.
16. Seilicovich A, Lasaga M, Befumo M *et al.* Nitric oxide inhibits the release of norepinephrine and dopamine from the medial basal hypothalamus of the rat. *Proc Natl Acad Sci USA* 1995; 92: 11299—11302.
17. Whitnall MH, Kiss A, Aguilera G. Contrasting effects of central alpha-1-adrenoreceptor activation on stress-responsive and stress-nonresponsive subpopulations of corticotropin-releasing hormone neurosecretory cells in the rat. *Neuroendocrinology* 1993; 58: 42—48.
18. Shioda S, Yada T, Muroya S, Takigawa M, Nakai Y. Noradrenaline activates vasopressin neurons via α_1-receptor-mediated Ca^{2+} signalling pathway. *Neurosci Lett* 1997; 226: 210—212.
19. Bugajski J, Gądek-Michalska A, Ołowska A, Borycz J, Głód R. Role of nitric oxide in the vasopressin-induced corticosterone secretion in rats. *J Physiol Pharmacol* 1997; 48: 805—812.
20. Bugajski J, Borycz J, Gądek-Michalska A, Głód R. Effect of L-NAME, a specific nitric oxide inhibitor, on corticotropin-releasing hormone-elicited ACTH and corticosterone secretion. *J Physiol Pharmacol* 1998; 49: 607—616.
21. Salter M, Duffy C, Garthwaite J, Strijbos PJLM. Substantial regional and hemispheric differences in brain nitric oxide synthase (NOS) inhibition following intracerebroventricular administration of Nω-nitro-L-arginine (L-NA) and its methyl ester (L-NAME). *Neuropharmacology* 1995; 34: 639—649.
22. Reif DW, McCreedy SA. N-nitro-L-arginine and N-monomethyl-L-arginine exhibit a different pattern of inactivation toward the three nitric oxide synthases. *Archiv Biochem Biophys* 1995; 320: 170—176.
23. Bugajski J, Gądek-Michalska A, Ołowska A, Borycz J, Głód R, Bugajski AJ. Adrenergic regulation of the hypothalamic-pituitary-adrenal axis under basal and social stress conditions. *J Physiol Pharmacol* 1995; 46: 297—312.
24. Brooks VL, Blakemore LJ. Vasopressin: a regulator of adrenal glucocorticoid production? *Am J Physiol* 1989; 256 (4 Pt 2): E566—E572.
25. Guillon G, Trueba M, Joubert D *et al.* Vasopressin stimulates steroid secretion in human adrenal glands: comparison with angiotensin-II effect. *Endocrinology* 1995; 136: 1285—1295.
26. Hinson JP, Vinson GP, Porter ID, Whitehouse BJ. Oxytocin and arginine vasopressin stimulate steroid secretion by the isolated perfused rat adrenal gland. *Neuropeptides* 1987; 10: 1—7.
27. Saito M, Sugimoto T, Tahara A, Kawashima H. Molecular cloning and characterization of rat V_{1b} vasopressin receptor: evidence for its expression in extra-pituitary tissues. *Biochem Biophys Res Commun* 1995; 212: 751—757.
28. Grazzini E, Lodboerer AM, Perez-Martin A, Joubert D, Guillon G. Molecular and functional characterization of V_{1b} vasopressin receptor in rat adrenal medulla. *Endocrinology* 1966; 137: 3906—3914.

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Author's address: Prof. Jan Bugajski, Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland, e-mail: bugajski@if-pan.krakow.pl.