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ROLE OF NITRIC OXIDE IN THE VASIOPRESSIN-INDUCED CORTICOSTERONE SECRETION IN RATS

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The presence of nitric oxide synthase (NOS) in hypothalamic structures which control the activity of the hypothalamic-pituitary-adrenal (HPA) axis suggests a role for NO in regulation of ACTH and corticosterone secretion. We investigated the involvement of NO in the corticosterone secretion induced by vasopressin (AVP), a potent coregulator of the HPA activity. AVP injectd ip was, on a molar basis, considerably more potent than administered interacerebroventricularly in inducing corticosterone secretion. This finding suggests a preferential action of AVP on pituitary corticotrop receptors, but not on central structures involved in stimulation of the HPA axis. Dexamethasone given before AVP totally abolished the AVP-elicited corticosterone response by a feedback mechanism and/or inhibition of the phospholipase A_2 activity and prostaglandin synthesis. Pretreatment with the NOS inhibitors L-NAME and L-NNA augmented significantly and to a similar extent the corticosterone response to AVP administered both systemically and centrally and L-NNA was found to be more potent in this respect. Pretreatment with L-arginine markedly reduced the AVP-induced corticosterone response. These results suggest that endogenous nitric oxide is significantly involved in the AVP-elicited corticosterone secretion and NO-induced alterations in the prostaglandin synthesis may participate in this action.

Key words: *nitric oxide, nitric oxide synthase antagonists, vasopressin, corticosterone, prostaglandins.*

INTRODUCTION

Vasopressin is a well documented coregulator of ACTH release. It is regarded to act synergistically with corticotropin releasing hormone (CRH) to produce an augmented release of ACTH (1). In the rat brain, AVP synthesized in the hypothalamic supraoptic paraventricular nuclei significantly contributes to activation of the pituitary-adrenal system. Parvicellular neurons produce both CRH and AVP, and vasopressin coexists in half of the corticotropin-releasing

hormone axons in the external zone of the median eminence in rats (2). Also AVP released from magnocellular axons during the passage through the internal zone of the median eminence may gain access to the pituitary portal circulation through fenestrated capillaries, or *via* vascular connections between the posterior and anterior lobes of the pituitary gland (1). However, the bulk of AVP in the hypophysial portal vessels is derived from nerve fibres in the median eminence.

We have found recently that AVP administered systemically is, on a molar basis, nearly as potent as CRH in stimulating the pituitary-adrenal axis, and that AVP can induce an almost maximal corticosterone secretion (3). Vasopressin given systemically activates directly its receptors on the anterior pituitary corticotrops and stimulates ACTH release. The potency and mechanism of action of the centrally administered AVP is not yet clear. We have also shown that the stimulatory action of AVP administered either systemically or intracerebroventricularly is significantly mediated by β-adrenergic and histamine receptors and prostaglandins (3—5).

Nitric oxide, an ubiquitous intra- and intercellular messenger, participates in several physiological functions in the CNS (6, 7). Nitric oxide synthase (NOS), an enzyme responsible for the synthesis of NO from its precursor L-arginine, is widely distributed in the CNS and is present at relatively high concentration in pericarya of the hypothalamus (8, 9). Nitric oxide synthase was found to be colocalized with magnocellular oxytocin (OT) and arginine vasopressin, as well as with parvocellular CRH. However, colocalization of NO in AVP and CRH positive cells was less frequent than in OT containing neurons (10). It has been shown that NO can stimulate centrally and control the release of vasopressin (11, 12). Endogenous NO is known to modulate the activation of the HPA axis by interleukin-1β, vasopressin and oxytocin (13—15).

In the present study we have examined the role of endogenous nitric oxide synthase in the HPA response to vasopressin. Effects of the specific inhibitors of NO synthase L-NAME and L-NNA, on corticosterone secretion elicited by systemic or intracerebroventricular AVP were assessed in conscious rats.

MATERIALS AND METHODS

Adult male Wistar rats, weighing 200—230 g, were housed in groups of 6 per cage in the animal room at a temperature of 21°C. They had free access to food and water and were kept under normal day-night lighting conditions. Drugs were dissolved in saline immediately before use, and were injected i.p. in a volume of 0.2 ml/kg or were administered into the right lateral cerebral ventricle in a volume of 10 μl to rats whose skulls were prepared one day earlier under light ether anesthesia for free-hand icv injections. Arginine-vasopressin was injected ip or intracerebroventricularly (icv). L-arginine and the NOS inhibitors Nω-nitro-L-arginine methyl

ester (L-NAME) and Nω-nitro-L-argnine (L-NNA) were administered ip 15 min before vasopressin. After injection of the drugs, the rats were placed back to their cages and decapitated 1 h later. Control animals received simultaneously 0.2 ml or 10 µl of saline, and were left undisturbed until decapitation concurrently with the animals injected with the drugs. One hour after the last injection, the rats were killed by rapid decapitation and their trunk blood was collected. After centrifugation, aliquots were frozen at −70°C until the assay. The concentration of corticosterone was measured fluorometrically. To avoid corticosterone fluctuations due to the circadian rhythm, all experiments were performed between 9—11 h, and all decapitations took place between 11—12 h. The drugs used: arginine vasopressin, the arginine derivatives that block the NO synthase activity, L-NAME and L-NNA, and the NO substrate-L-arginine were purchased from Sigma.

Statistical analysis. All results are expressed as mean ± SEM. Statistical probabilities were calculated by an analysis of variance, followed by individual comparisons with the Duncan test. A probability value of <0.05 indicated a statistically significant difference between the group means.

RESULTS

Effect of vasopressin on corticosterone secretion

Vasopressin injected ip or icv elicited a significant, dose-related increase in serum corticosterone levels measured 1 h later. On a molar basis, AVP given ip was considerably more potent in inducing the HPA response than when given icv (*Fig. 1*). The synthetic glucocorticoid dexamethasone (0.2 mg/kg) which has a high affinity for the Type 2 glucocorticoid receptor, given ip 1 h before AVP (5 µg/kg ip), almost totally abolished, by 93%, the AVP-induced corticosterone response (*Fig. 2*).

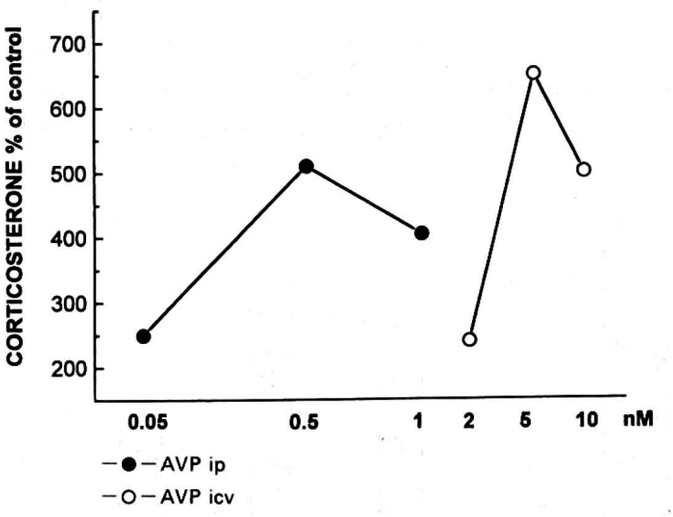

Fig. 1. Corticosterone secretion after ip and icv administration of vasopressin (AVP), log dose-response values. 1 h after AVP injection the rats were decapitated. Values represent the mean ± SEM of 7 rats.

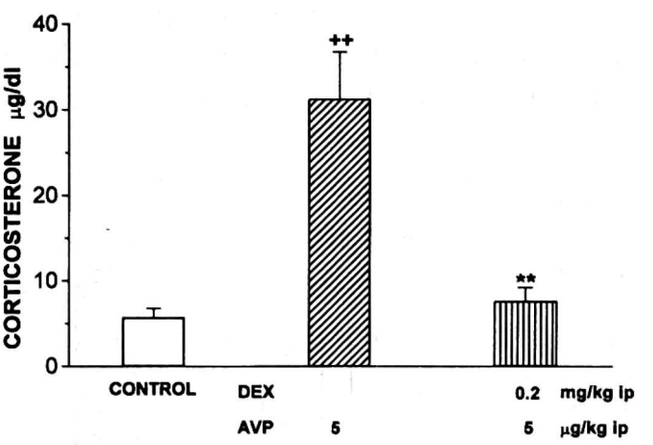

Fig. 2. Effect of dexamethasone (DEX) on AVP-induced corticosterone secretion. DEX was injected 1 h before AVP. In *Fig. 2—6* values represent the mean ± SEM of 6—7 animals. *$p < 0{,}05$ and **$p < 0{,}01$ *vs.* saline control; *$p < 0{,}05$ and **$p < 0{,}01$ *vs.* AVP-treated group.

Effect of nitric oxide synthase antagonists on the AVP-induced corticosterone response

Neither the NOS blockers L-NAME and L-NNA, nor L-arginine given alone in doses chosen for the present study measurably altered the basal serum corticosterone levels (results not shown). L-NAME (2—20 mg/kg), an inhibitor of endogenous nitric oxide synthase, given ip 15 min prior to AVP (5 μg/kg) significantly augmented the corticosterone response to AVP given by the same route. The most pronounced increase, by 104% above evoked by AVP was induced by L-NAME given in a dose of 10 mg/kg (*Fig. 3*). A similarly strongest rise in the AVP-induced corticosterone secretion, by 94%, was elicited by L-NNA administered in a smaller dose of 2 mg/kg (*Fig. 4*). The NOS synthase blocker L-NAME (5 mg/kg ip) also significantly raised, by 51%, the corticosterone secretion enhanced by AVP administered icv (*Fig. 5*). In contrast, L-arginine (20 mg/kg ip) considerably decreased the AVP-elicited increase in corticosterone secretion (*Fig. 6*).

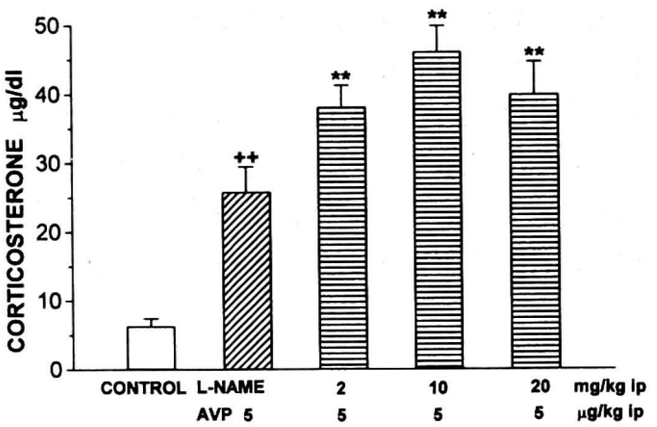

Fig. 3. Effect of L-NAME on AVP-induced corticosterone secretion. L-NAME was injected ip 15 min before AVP. See legend to *Fig. 2.*

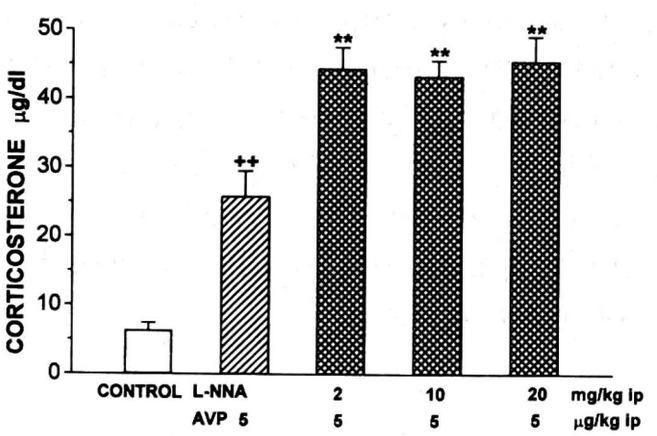

Fig. 4. Effect of L-NNA on AVP-induced corticosterone secretion. L-NNA was injected 15 min prior to AVP. See legend to *Fig. 2.*

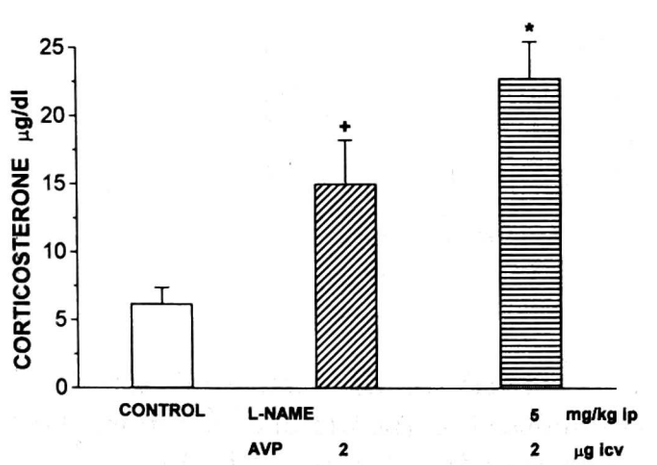

Fig. 5. Effect of L-NAME on icv AVP-induced serum corticosterone levels. L-NAME was injected ip 15 min prior to AVP. See legend to *Fig. 2.*

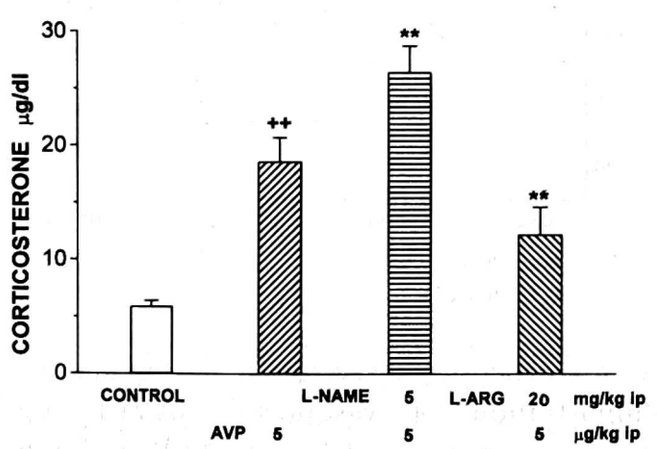

Fig. 6. Effect of L-NAME and L-arginine on AVP-induced serum corticosterone levels. L-NAME and L-ARG were injedted ip 15 min before AVP. See legend to *Fig. 2.*

DISCUSSION

The present results confirm our former data that AVP given alone systemically increases dose-dependently the HPA response, measured indirectly through corticosterone secretion. AVP given ip has ready access to its receptors on anterior pituitary corticotrops and it induces ACTH release which in turn stimulates corticosterone secretion. AVP given ip may penetrate the blood-brain barrier and indirectly stimulate hypothalamic structures involved in activation of the HPA axis. This possibility seems unlikely, since icv administered AVP induced a far weaker stimulatory effect compared to that after systemic injection. AVP given icv moderately decreased the hypothalamic noradrenaline level, and the AVP-induced increase in corticosterone secretion was significantly diminished by propranolol, a β-adrenergic antagonist (3), though it did not influence the hypothalamic content of AVP under normal conditions (16). Also hypothalamic histamine, as well as histamine H_1 and H_2 receptors significantly mediated the stimulatory effect of ip AVP (4), but not of AVP injected centrally (data not shown). However, indirect stimulation of the hypothalamic CRH release by a central adrenergic or hstaminergic system seems unlikely to be a major mechanism of the icv AVP-induced corticosterone response. Rather part of the icv AVP may reach the anterior pituitary via portal circulation and may directly stimulate the release of ACTH from pituitary corticotrops and subsequently corticosterone secretion. These results also indicate that direct stimulation by AVP of corticosterone secretion from adrenal glands *in vivo* is unlikely, though it was observed in the perfused rat adrenal gland and cultured zona fasciculata cells (17, 18).

Pretreatment with dexamethasone (0.2 mg/kg ip 1 h before AVP) completely abolished the AVP-induced corticosterone response. Dexamethasone is known to exert negative feedback control of the anterior pituitary ACTH secretion induced by a variety of secretagogues, *via* occupation of intracellular steroid receptors and subsequent modulation of protein synthesis. In our experiment, inhibition of ACTH and corticosterone secretion took place in an early delayed feedback phase (2 h after dexamethasone injection) which depends on *de novo* generation of protein second messengers, probably lipocortin 1 and phospholipid binding protein, which inhibit the release rather than the synthesis of ACTH (19).

Like glucocorticoids, dexamethasone, inhibits the activity of phospholipase A_2 and thus reduces the release of arachidonic acid and ultimately inhibits the formation of prostanoids. Although dexamethasone affects the inducible but not constitutive cyclooxygenase expression (20), the former (COX-2) is considered to be the dominating COX isoform in the brain (21). Reduction by dexamethasone of the AVP-elicited corticosterone response in the present experiment may result from the suppresion of PG synthesis, since pretreatment

with indomethacin abolished the AVP-induced corticosterone secretion under both basal and stress conditions in our recent investigation (5, 22). Mutual involvement of the feedback mechanism and/or the reduction of PLA_2, as well as the PG release in the AVP-induced pituitary-adrenocortical response by dexamethasone observed in the present experiment, need further elucidation.

The obtained results clearly show that endogenous NO significantly affects the AVP-induced pituitary-adrenal activity. Inhibition of the endogenous NO synthesis by the NOS blockers L-NAME and L-NNA considerably augments the AVP-elicited corticosterone response. Our results indicate that L-NNA is more potent than L-NAME in this respect, since maximal enhancement of the AVP-evoked corticosterone response was induced by ip pretreatment with 2 and 10 mg/kg of those blockers, respectively. This phenomenon may be due to a slower rate of transport across the blood-brain barrier of L-NAME which is a weak inhibitor of neuronal NOS and must be first deestrified to L-NNA to potently inhibit n-NOS (23).

L-NAME in a large dose (30 mg/kg iv) also raised plasma corticosterone to a nearly maximal level (15). According to some authors, the ability of the systemically administered AVP to stimulate ACTH secretion in the anterior pituitary, may by greatly enhanced by endogenous CRH (24). In this case, a significant increase in the AVP-induced corticosterone secretion elicited by L-NAME may partly be due to the stimulation of CRH release by this NOS blocker (13). In the present study L-NAME also significantly augmented the corticosterone response to icv AVP. This finding indicates that inhibition of the NO synthesis induces a similar stimulatory effect which depends on neither the route of administration nor the site of action of AVP. The inhibitory effect of endogenous NO on the AVP-induced corticosterone response was confirmed by the fact that L-arginine, a substrate for NO, potently, by 50%, diminished the AVP-elicited corticosterone response.

Nitric oxide may affect the HPA response to AVP *via* an interaction with the cyclooxygenase pathway. At higher concentrations, NO may indirectly stimulate cyclooxygenases and subsequentyl increase the synthesis of prostaglandins shown in cultured endothelial cells (25), pancreatic islets (26) and the rat skin vasculature (27). However, NO is able to potently reduce prostanoid synthesis in the kidney (28). We have found that prostaglandins significantly mediate the AVP-induced corticosterone response (5, 22). These findings strongly suggest involvement of PGs in the AVP-induced changes in corticosterone responses evoked by L-arginine and NOS blockers in the present experiment. It has also been found that NO inhibits, while PGs stimulate the ACTH response to local inflammation, mediated by the synergistic action of AVP and CRH (29).

In rat hypothalamic explants *in vitro,* NO directly inhibited stimulated release of CRH (13). However, *in vivo* NO functions as a unique mechanism of

the synaptic and inter-cellular system of communication. Our present and former results (5, 22) suggest that in the AVP-induced HPA response, NO is able to interact with both PGs and the neurotransmitter systems that mediate the AVP response.

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