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CATECHOLAMINERGIC REGULATION OF THE HYPOTHALAMIC-PITUITARY-ADRENOCORTICAL ACTIVITY

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The significance and site of adrenergic receptors involved in the control of the hypothalamic-pituitary-adrenal axis (HPA) activity was assessed indirectly by estimation of serum corticosterone levels 1 h after drug administration to conscious rats. Adrenergic drugs were given intracerebroventricularly (icv) and intraperitoneally (ip), the antagonists 15 min prior to the agonists. Noradrenaline, adrenalin and isoproterenol given by either route increased dose-dependently the serum corticosterone levels. The corticosterone response to icv noradrenaline was almost abolished by icv pretreatment with propranolol, a β-adrenergic antagonist, and yohimbine, an α_2-receptor blocker, and was also considerably reduced by prazosin, an α_1-adrenergic antagonist. When given ip, these antagonists did not significantly influence the noradrenaline induced corticosterone response, which suggests a suprapituitary site of action of noradrenaline in stimulation of the HPA. The corticosterone response to icv adrenalin was suppressed by prazosin given by either route. The corticosterone response to ip adrenalin was almost abolished by pretreatment with yohimbine, and also significantly diminished by propranolol given by the same route. The increase in corticosterone secretion, induced by isoproterenol given by either route, was abolished by ip injection of propranolol.
These results indicate that noradrenaline stimulates the HPA via α- and β-adrenergic receptors, mainly at the suprapituitary level. Adrenalin increases that activity both via central and pituitary α- and β-adrenoceptors. Isoproterenol activates the HPA by stimulation of pituitary β-receptors.

Key words: *catecholamices, adrenergic antagonists, pituitary-adrenocortical activity, corticosterone.*

INTRODUCTION

Pharmacological and biochemical studies demonstrated that catecholamines play an important role in regulation of the secretion of most of the hypophyseotropic hormones and other neuropeptides in the hypothalamus (1, 2). The release of proopiomelanocortin-derived peptides from the ante-

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rior pituitary lobe is regulated by the hypothalamic releasing factor CRF, and may also be influenced by catecholamines (3—7). Until recently a number of physiological and pharmacological arguments favoured the theory of the inhibitory role of centrally acting catecholamines in the CRF release. Various in vivo and in vitro approaches using agonists and antagonists suggested that catecholamines inhibit CFR-ACTH and corticosterone secretion (8-9). However, in the past few years an increasing body of evidence has accumulated in favour of the stimulatory effect of both noradrenaline and adrenalin on the CRF secretion (2, 5, 10—14). A vast body of evidence indicates that catecholamines may stimulate directly ACTH release at the pituitary level, possibly as a synergist with CRF (3, 6, 15). Both α- and β-adrenergic receptors mediate ACTH secretion from the anterior and intermediate lobes of the pituitary (3, 15-17) However, at the hypothalamic level the action of catecholamines and the involvement of adrenergic receptors in regulation of CRF secretion remain controversial.

In the present study we examined the effects of central and systemic administration of catecholamines on the hypothalamic-pituitary-adrenocortical activity. The involvement of particular types and sites of adrenergic receptors in those effects was also studied.

MATERIAL AND METHODS

Male Wistar rats, weighing 180—220 g, were housed in groups of 7 per cage, and maintained with commercial food and drinking water ad libitum on the diurnal light cycle at an ambient room temperature of 18—21°C one week before the experiment. The animals were arbitrarily assigned to one of the experimental groups. The drugs contained in 10 μl of saline were injected into the right lateral cerebral ventricle of non-anesthetized rats (18) and were dissolved in a volume of 1 ml/kg for intraperitoneal injection. Control rats received 0.9% NaCl solution, 10 μl or 0.2 ml, respectively. Adrenergic antagonists were injected 15 min before adrenergic agonists. After injection of the drugs the animals were placed back in their cages. Although the serum corticisterone concentration was significantly elevated by icv or ip saline injection at 15—45 min, it declined to a basically resting level up till 60 min. Therefore in further experiments the drugs were injected 60—75 min before the rats were killed. The rats were decapitated immediately after their removal from cages and their trunk blood was collected. The control animals were decapitated concurrently with the experimental group to obtain resting serum corticosterone levels. After centrifugation serum aliquts were frozen until the assay. The serum corticosterone concentration was determined fluorometrically (18). Serum corticosterone levels are expressed as micrograms per 100 ml. One analysis was performed in each rat's serum, but 6—16 animals were used for every data point. In order to avoid interference with the circadian rhythm in corticosterone levels, all decapitations were carried out between 10 and 11 a. m., i.e. when the serum corticosterone concentration is low in the normal diurnal rhythm.

The following drugs were used: (—)arterenol bitartrate (norepinephrine bitartrate), L-epinephrine bitartrate (adrenalin bitartrate), DL-isoproterenol HCl, yohimbine hydrochloride (Sigma), prazosin (Pfizer). All the drug solutions were prepared in sterile saline immediately before use. The data are presented as arithmetical means and standard errors of the means. The significance of differences between groups was assesed by an unpaired t-test.

RESULTS

Effects of catecholamines on the serum corticosterone levels

Exogenous catecholamines, noradrenaline and adrenalin, administered icv to conscious rats, induced a significant dose-related increase in serum corticosterone levels measured 1 h after administration. Adrenalin was somewhat stronger in this respect. When injected ip, those drugs also induced

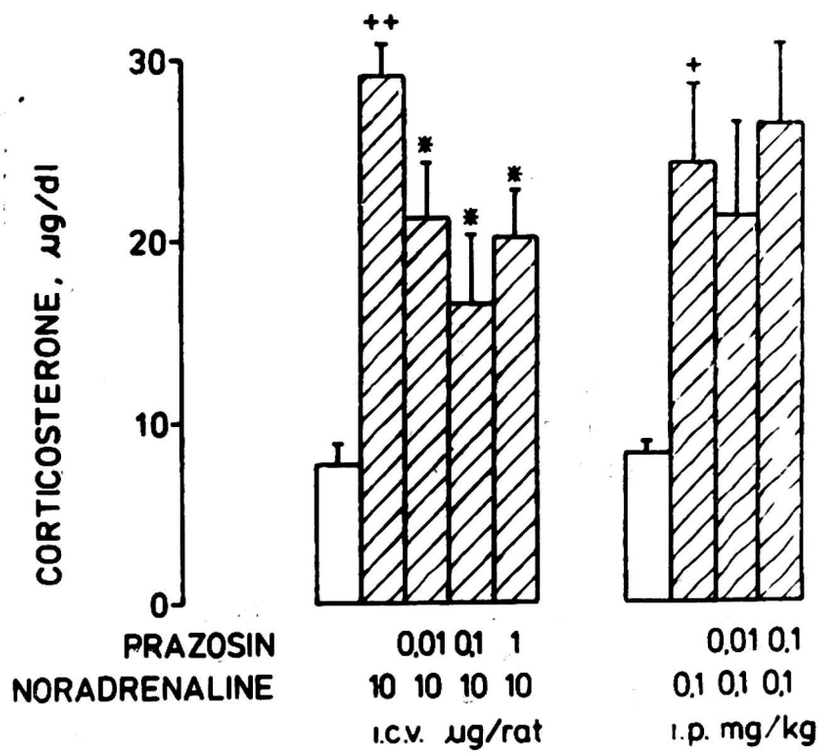

Fig. 1. Effect of prazosin on noradrenaline-induced rise in serum corticosterone concentration in rats. The drugs were injected icv or ip, prazosin 15 min before noradrenaline and 1 h later the rats were decapitated. Each value represents the mean ±SEM of 6—13 rats. $+p < 0.05$ and $++p < 0.001$ vs. saline-treated controls; $*p < 0.05$ vs. noradrenaline treated group.

a significant rise in the serum corticosterone levels as compared with control saline-injected rats.

To antagonize the effect of treatment with catecholamines, adrenergic receptor blockers were used in doses which by themselves had no significant hormonal effect.

Effect of adrenergic antagonists on the noradrenaline-induced corticosterone response

Prazosin, an α_1-adrenergic receptor antagonist injected ivc 15 min prior to noradrenaline given by the same route considerably decreased, by 59%, the corticosterone response to this adrenergic agonist. This suggests a hypothalamic site of action of noradrenaline on HPA axis.

When given ip prazosin did not influence markedly the increase in serum

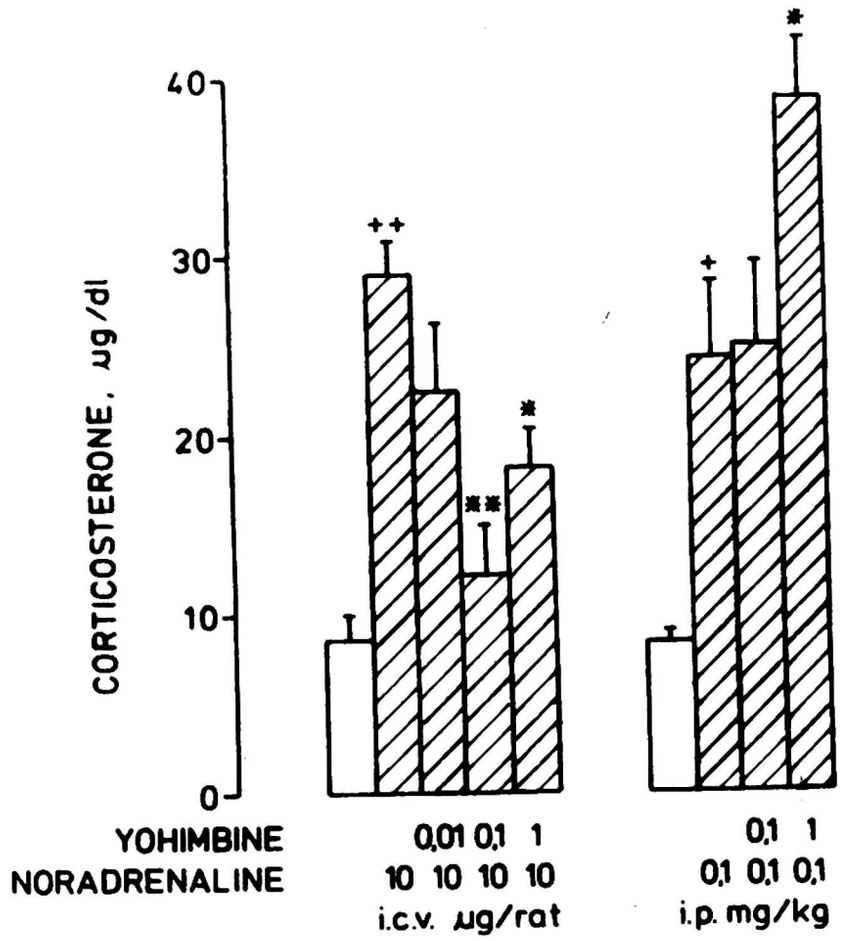

Fig. 2. Effect of yohimbine on noradrenaline-induced corticosterone response. Yohimbine was given 15 min before noradrenaline. Each value represents the mean ±SEM of 7—12 rats. $+p < 0.05$ and $++p < 0.001$ vs. saline treated controls; $*p < 0.05$ and $**p < 0.001$ vs. noradrenaline treated group.

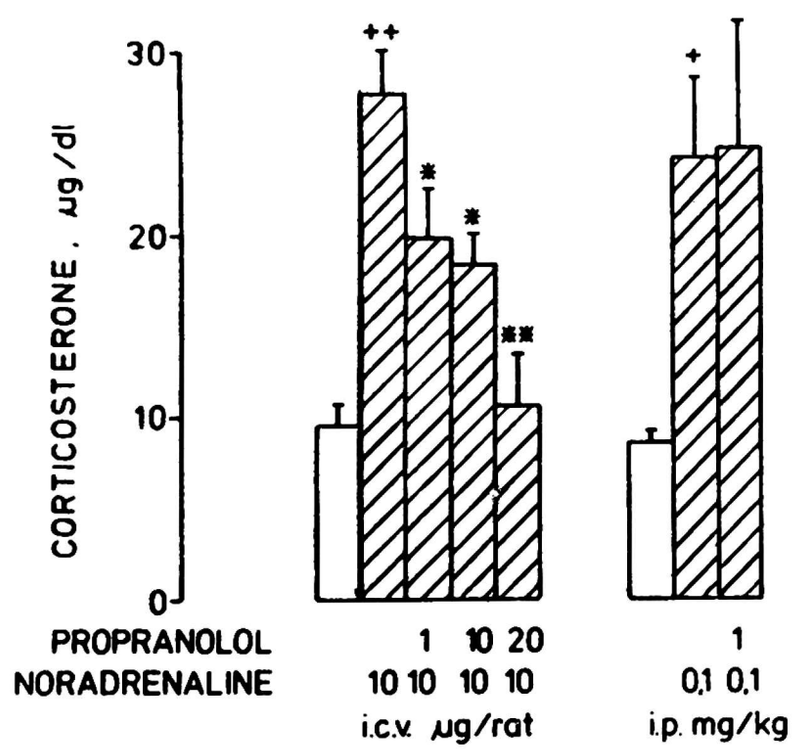

Fig. 3. Effect of propranolol on noradrenaline-induced corticosterone response. Propranolol was given 15 min before noradrenaline. Each value respresents the mean ±SEM of 6—13 rats. $+p < 0.05$ and $++p < 0.001$ vs. saline treated controls; $*p < 0.05$ and $**p < 0.001$ vs. noradrenaline treated group.

corticosterone levels induced by noradrenaline given ip (*Fig. 1*), which suggests that the pituitary is not a significant site of action of systemically injected noradrenaline.

Intraventricular pretreatment with yohimbine, an α_2-adrenergic antagonist, almost totally suppressed the corticosterone response to icv noradrenaline, indicating a considerable involvement of hypothalamic α_2-adrenoceptors. Yohimbine given ip in a small dose did not impair, while in a larger dose it significantly increased the noradrenaline-induced rise in serum corticosterone level (*Fig. 2*).

Like prazosin and yohimbine, the β-adrenergic antagonist propranolol was able to inhibit totally the corticosterone response to noradrenaline when both those drugs were given icv, but it had no effect on the corticosterone response when it was injected ip before noradrenaline given by the same route (*Fig. 3*).

Effect of adrenergic antagonists on the adrenalin-induced corticosterone response

Intraventricular pretreatment with prazosin almost totally abolished the rise in serum corticosterone levels induced by adrenalin when both those drugs were administered icv. Prazosin also significantly, by 47%, impaired

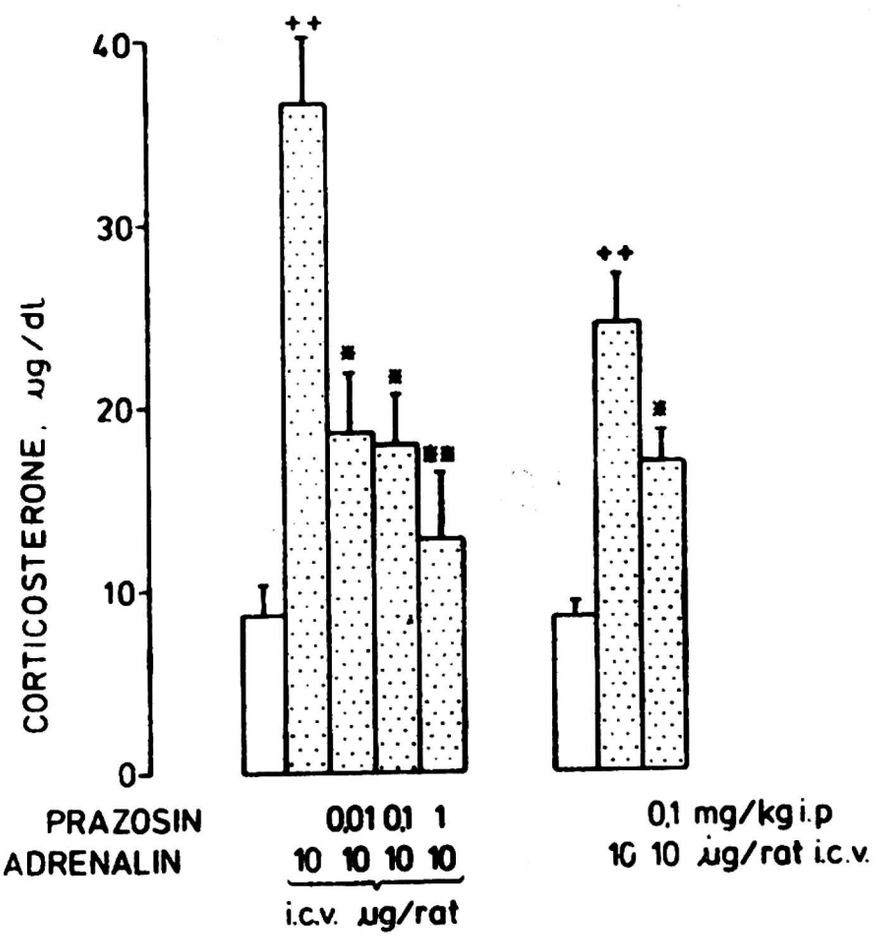

Fig. 4. Effect of prazosin on adrenalin-induced corticosterone response. Prazosin was given 15 min before adrenalin. Each value respresents the mean ±SEM of 7—13 rats. ++p < 0.001 vs. saline treated controls; *p < 0.05 and **p < 0.001 vs. adrenalin treated group.

the corticosterone response when it was injected ip before adrenalin (*Fig. 4*).

Yohimbine markedly diminished, by 60%, the rise in serum corticosterone levels when it was injected ip 15 min before adrenalin given by the same route (*Fig. 5*).

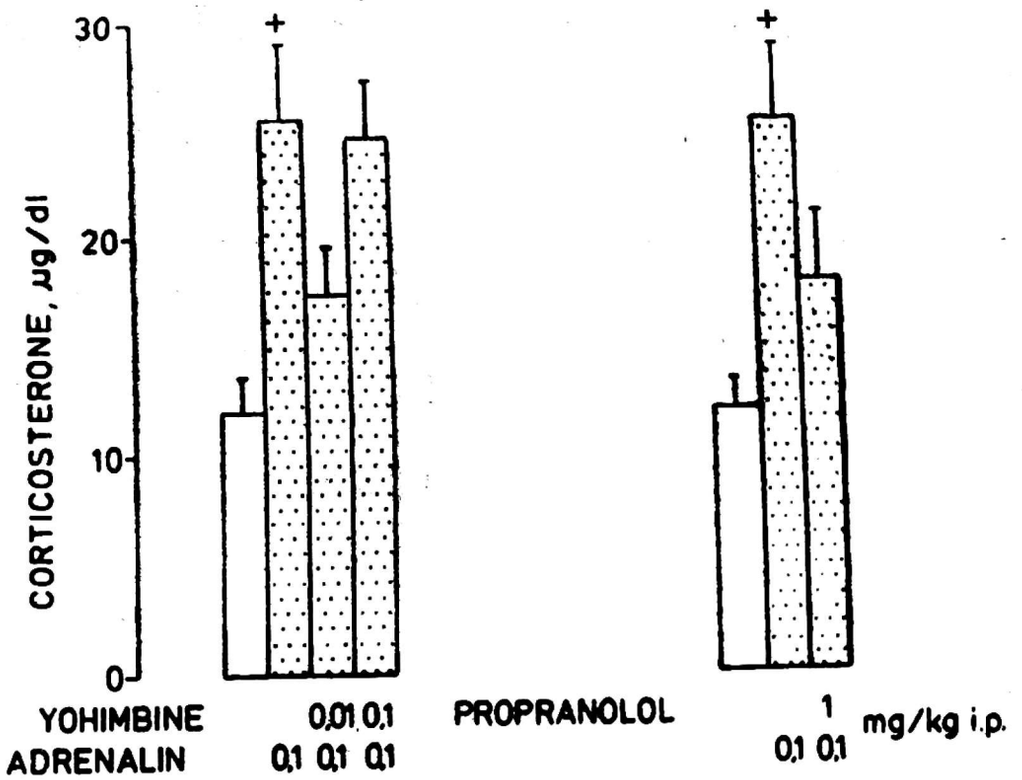

Fig. 5. Effect of yohimbine and propranolol given ip on serum corticosterone response to adrenalin. The antagonists were given 15 min before adrenalin. Each value represents the mean ±SEM of 7—12 rats.

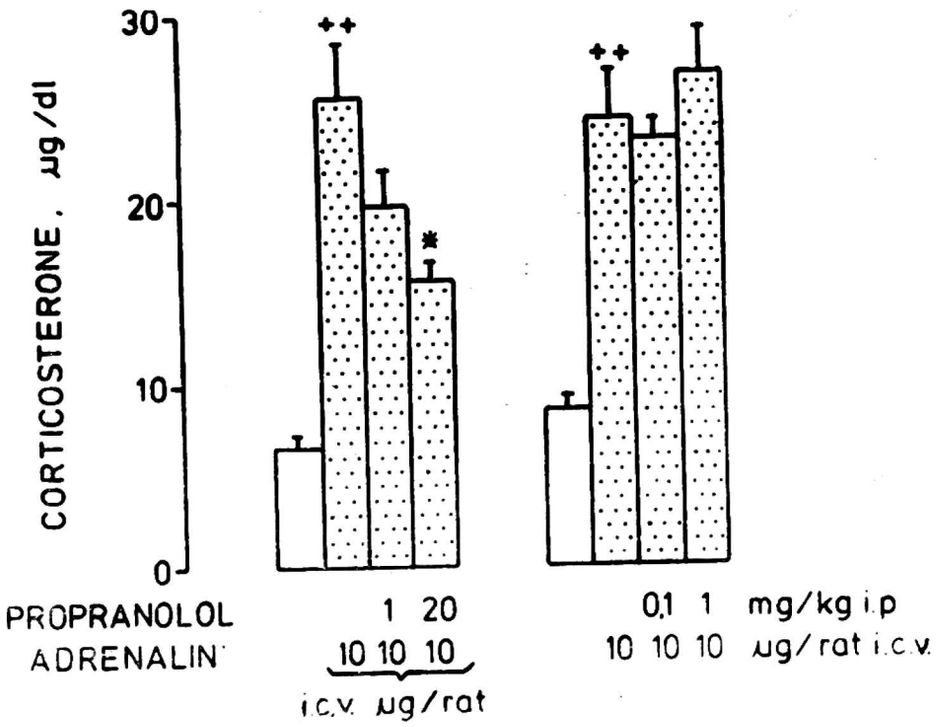

Fig. 6. Effect of propranolol on adrenalin-induced corticosterone response. Propranolol was given 15 min before adrenalin. Each value represents the mean ±SEM of 6—12 rats. $++p < 0.001$ vs. saline treated controls; $*p < 0.05$ vs. adrenalin treated group.

The increase in the the serum corticosterone evoked by centrally administered adrenalin was considerably reduced, by 55%, by propranolol given in larger doses both icv and ip, 20 μg and 1 mg/kg, respectively. On the other hand, ip propranolol did not influence the rise in the serum corticosterone induced by central injection of adrenalin (*Fig. 6*). This suggests that adrenalin may stimulate CRF containing neurons, at least in part, via β-receptors within the hypothalamus and also β-adrenoceptors at the pituitary corticotrophs.

Effect of adrenergic antagonists on the isoproterenol-induced corticosterone response

Neither prazosin nor yohimbine given ip 15 min before isoproterenol antagonized the rise in serum corticosterone levels induced by icv administration of that β-adrenergic receptor agonist (data not shown). Only propranolol given ip totally abolished the increase in serum corticosterone levels evoked by icv administration of isoproterenol (*Fig. 7*).

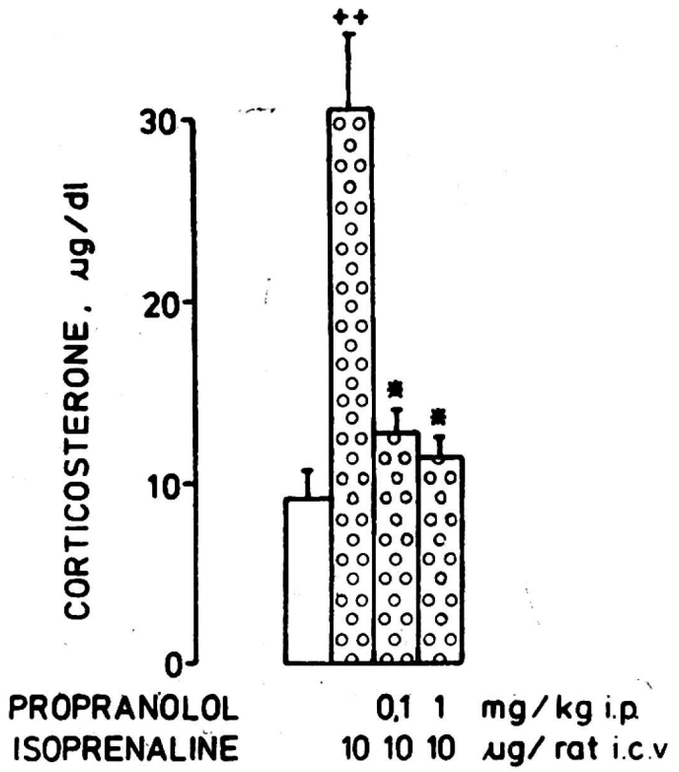

Fig. 7. Effect of propranolol on isoproterenol-induced corticosterone response. Propranolol was given 15 min before isoproterenol. Each value represents the mean ±SEM of 12 rats. ++$p < 0.001$ vs. saline treated controls; *$p < 0.05$ vs. isoproterenol treated group.

DISCUSSION

In the present study catecholamines noradrenaline, adrenalin and isoproterenol given icv to unanesthetized rats considerably increased the pituitary-adrenocortical activity. After icv administration these drugs are likely

to penetrate into the hypothalamic structures and directly act on adrenergic neurons in the parvocellular part of the hypothalamic paraventricular nucleus which contains CRF producing cells (2, 10, 14, 19). Recently a direct synaptic noradrenergic connections with these cells have been demonstrated (4). Strong stimulation of the HPA axis by catecholamines given icv, observed in the present experiment, may suggest activation of adrenergic receptors on these neurons.

It is well known that, when injected systemically, catecholamines do not easily cross the blood-brain barrier. Their stimulatory effect on the corticosterone secretion, found in the present experiment, may be due to either a direct action on the hypophysis or median eminence, which are not protected by the blood-brain barrier, or to an indirect peripheral stimulation, transmitted to the hypothalamus.

Although the drugs in question given icv may also reach the hypophysis via portal circulation and liberate ACTH by stimulation of α-and β-adrenoceptors, present on corticotrophs in the anterior hypophysis, such a possibility seems unlikely, since in the present experiment systemic administration of adrenergic blockers did not antagonize the corticosterone response to noradrenaline as it was observed after icv administration.

The present results show that the HPA stimulation by noradrenaline given centrally was abolished or considerably diminished by prior administration of propranolol, yohimbine or prazosin, β-, α_2- or α_1-receptor antagonists, respectively. Pretreatment with prazosin only slightly decreased, whereas propranolol administration did not change the corticosterone response to noradrenaline when those drugs were given systemically. On the other hand in our current experiment prazosin antagonized the corticosterone secretion induced by ip phenylephrine, an α_1-adrenergic agonist, suggesting a pituitary site of action (20). In the HPA stimulation by icv noradrenaline we observed unexpectedly powerful involvement of central α_2-adrenoceptors which was only described in cultured anterior pituitary lobe cells (21). The significant increase in the corticosterone response to noradrenaline in rats pretreated ip with a larger dose of yohimbie in the present experiment might de due to the known rise in the corticosterone levels evoked by yohimbine itself when larger doses are used (22, 23). These results give no satisfactory explanation of why α- and β-adrenergic antagonists given ip do not antagonize the effect of noradrenaline if, according to other data, both α_1- and β-adrenergic receptors are present on pituitary corticotrophs (3, 16, 17, 24).

The corticosterone response to icv adrenalin was strongly reduced, up to 85%, by icv pretreatment with prazosin, α_1-adrenergic antagonist, given icv and also significantly diminished, by 47%, after ip pretreatment with that drug. Since prazosin does not readily cross the blood-brain brarrier (25) these results suggest that during the HPA stimulation by adrenalin an

α_1-adrenergic mechanism prevails in the hypothalamus, being somewhat weaker at the pituitary level. The corticosterone response to icv adrenalin was significantly impaired by propranolol given icv and systemic pretreatment with propranolol also markedly diminished the corticosterone response to ip adrenalin. Our results suggest that in the stimulation of CRF and ACTH by icv adrenalin are involved α_1- and β-adrenoceptors of the hypothalamus and pituitary, in agreement with other findings (26—28). After systemic administration the rise in serum corticosterone levels evoked by adrenalin was also markedly decreased by yohimbine which points to partial mediation of α_2-adrenoceptors in the hypothalamus since α_2-adrenoceptors were not found on pituitary corticotrophs.

The stimulatory effect of isoproterenol administered icv on corticosterone secretion was totally abolished by ip pretreatment with propranolol which indicates the β-adrenoceptor mediated stimulation of the HPA by this β-adrenergic receptor agonist. Our results show that isoproterenol fairly selectively stimulates the HPA via β-adrenoceptors since neither prazosin nor yohimbine, α_1- and α_2-adrenergic receptor antagonists, respectively, influenced the corticosterone response induced by isoproterenol.

In conclusion, the present results indicate that noradrenaline stimulates the HPA via β-, α_2- and α_1-adrenergic receptors, mainly at the suprapituitary level. Adrenalin increases that activity via both central and pituitary α- and β-adrenoceptors. Isoproterenol activates the HPA by a selective stimulation of the pituitary and/or hypothalamic β-receptors. These data give support to the concept that central catecholaminergic systems are excitatory upon the CRF-ACTH secretion when they act directly at the hypothalamic level. The circulating adrenalin is also capable of stimulating directly the pituitary corticotrophs by activation of α- and β-adrenoceptors.

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