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EFFECT OF GAMMA-AMINOBUTYRIC ACID AND MUSCIMOL ON CORTICOSTERONE SECRETION IN RATS

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The effect of γ-aminobutyric acid-receptor agonists, GABA and muscimol on the pituitary-adrenocortical activity, measured indirectly through corticosterone secretion, and the receptors involved were investigated in conscious rats. GABA given ip induced a dual effect, in lower dose (10 mg/kg) it significantly decreased the resting serum corticosterone levels while in higher doses (100—500 mg/kg) it considerably raised that level. Muscimol (0.5 mg/kg ip) also increased the corticosterone concentration. Both GABA and muscimol given intracerebroventricularly (icv) induced a significant, dose-related increase in serum corticosterone levels. Bicuculline, a $GABA_A$-receptor antagonist, totally abolished the corticosterone response to GABA but did not influence the response to muscimol. Pretreatment with atropine did not affect the corticosterone response to GABA but significantly diminished the response to muscimol.

These results suggest that GABA moderately inhibits the pituitary-adrenal axis at the pituitary level but significantly stimulates it at the hypothalamic level. The stimulatory effect of GABA, but not muscimol, is mediated by hypothalamic $GABA_A$-receptors, and in the effect of muscimol hypothalamic cholinergic, muscarinic receptors are involved to a significant extent.

Key words: *GABA, muscimol, $GABA_A$-receptors, muscarinic receptors, pituitary-adrenocortical activity, corticosterone.*

INTRODUCTION

GABA has been implicated in modulating secretion of pituitary hormones and hypothalamic-hypophysial hormones. GABA-ergic neurocrine modulation may occur in the median eminence of hypothalamus, where GABA-ergic terminals are present, or directly at the pituitary level. Measurable amounts of

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endogenous GABA have been reported in the anterior pituitary lobe. GABA is synthesized in the neurointermediate lobe of the hypophysis in vitro, where glutamic acid decarboxylase activity has been found, but the anterior lobe is devoid of any positive immunoreactivity (1, 2). Therefore GABA in the anterior lobe of the pituitary appears to be of hypothalamic origin. $GABA_A$ receptors similar to those of the central nervous system and coupled with benzodiazepines and Cl– ionophores have been localized in the anterior pituitary and on axons of hypothalamic-neurophypophysial neurons (3, 4). GABA from the median eminence can be carried through the hypophyseal portal vessels to reach specific receptor sites located in the anterior pituitary (5). Portal plasma contains slightly or significantly higher concentration of GABA receptor ligands than peripheral plasma (6, 7). Electrical stimulation of median eminence evoked a 8-fold increase in the rate of release of GABA into portal blood, which suggests that GABA could be involved in the central control of anterior pituitary function.

GABA has an inhibitory effect on central thyrotropin control by inhibition of TRH release from hypothalamus (8) and inhibits the release of α-MSH from rat hypothalamic slices (9). Recent evidence supports the existence of a dual GABA-ergic control of prolactin secretion in the rat, one stimulatory exerted on a central nervous system site, and other inhibitory, occuring at the level of anterior pituitary (10, 11).

The neurotransmitter role of GABA in the central regulation of CRF and ACTH secretion is not clear at present. GABA has been reported to stimulate (12), to inhibit (13) and not to affect the hypothalamic-pituitary-adrenal axis activity.

This study was designed to determine the role and site of GABA receptors in control the pituitary-adrenocortical activity in the rat.

MATERIAL AND METHODS

Male Wistar rats, weighing 180—200 g, were housed in groups of 7 per cage, and maintained with commercial food and drinking water ad libitum on a diurnal light cycle at the ambient temperature of 18—21° C one week prior to experimentation. The animals were arbitrarily assigned to one of experimental groups. The drugs contained in 10 µl of saline were injected into the right lateral cerebral ventricle of non-anesthetized rats and for intraperitoneal injection they were dissolved in a volume of 1 mg/kg. Control rats received 0.9% NaCl solution, 10 µl or 0.2 ml, respectively. The GABA antagonist bicuculline was injected 15 min before GABA or muscimol. After injection of the drugs animals were placed back in their cages. Although the serum corticosterone concentration was significantly raised by the icv or ip saline injection at 15—45 min, it decreased to a basically resting level by 60 min.

Therefore in further experiments the drugs were injected 60—75 min before the rats were killed. The rats were decapitated immediately after the removal from cage and their trunk blood was collected. Control animals were decapitated concurrently with the experimental group to obtain resting serum corticosterone levels. After centrifugation serum aliquots were frozen until the assay. The serum corticosterone concentration was determined fluorometrically (14). Corticosterone levels in the serum was 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 by the circadian rhythm in corticosterone levels all the experiments were performed between 9 and 10 a.m., and all decapitations were carried out between 10 and 11 a.m. when serum corticosterone concentration is low in the normal diurnal rhythm (15).

Drugs used were: γ-aminobutyric acid (GABA), muscimol, bicuculline, carbamylcholine chloride (Sigma). The drugs were dissolved in a 0.9% NaCl solution immediately before use and bicuculline was dissolved in an acidified saline (1 N HCl) and buffered to pH 4.5 with 1 N NaOH.

The results were calculated as the group mean $\pm$ standard error of the mean. Statistical evaluation was performed by analysis of variance, followed by individual comparisons with the Duncan's test.

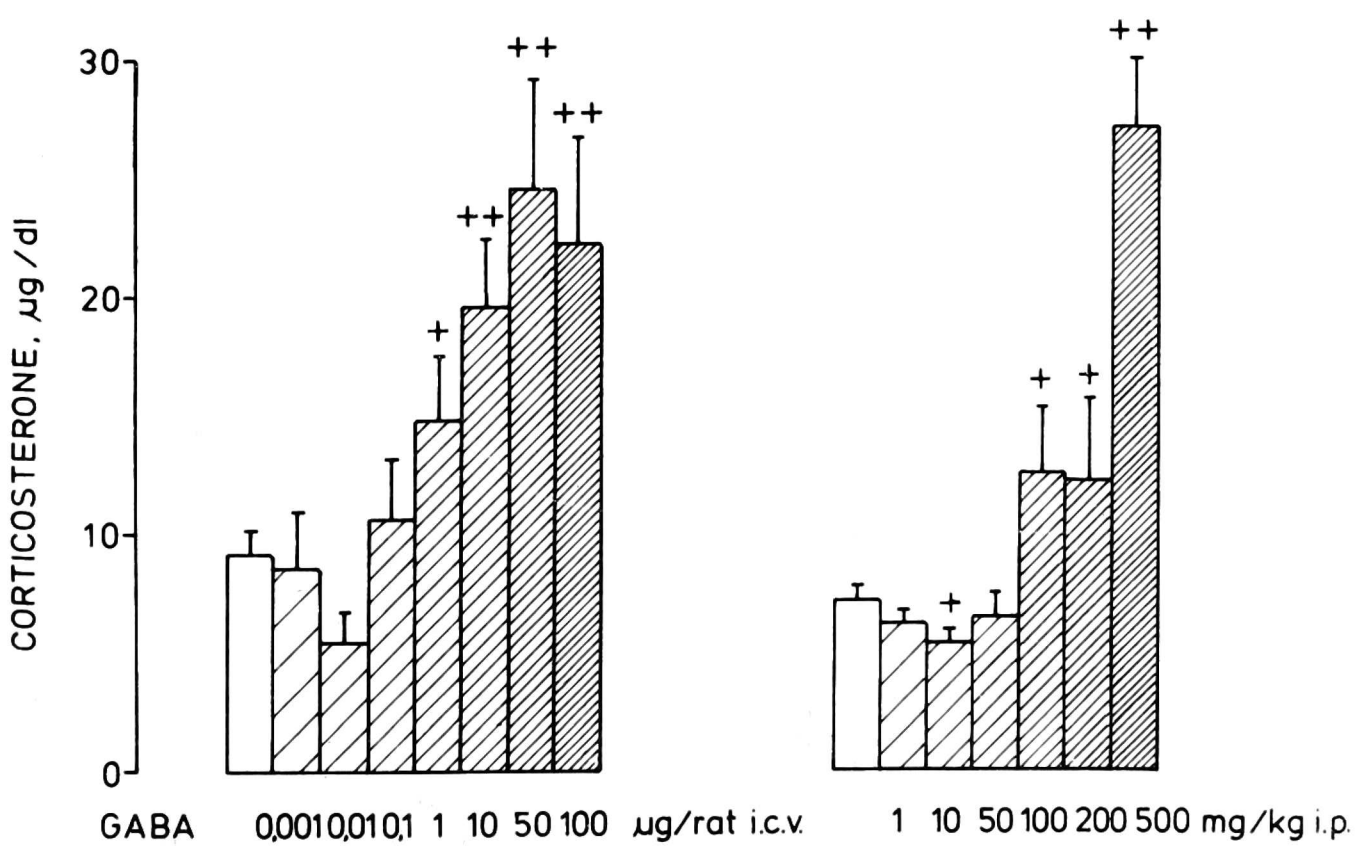

Fig. 1. Serum corticosterone concentrations in conscious rats 1 h after ip and icv GABA administration. Bars represent means, vertical lines SEM of 7—14 animals in each group. + $p<0.05$ and ++ $p<0.001$ vs. saline-treated group.

RESULTS

Effect of GABA and muscimol on corticosterone secretion

In order to assess a possible role of GABA receptors on the pituitary corticotrophs and on hypothalamic CRF containing neurons, GABA and muscimol, $GABA_A$-receptor agonists, were administered both systemically and intracerebroventricularly. GABA (1—100 μg) administered icv induced dose-related and significant increase in the serum corticosterone levels, measured 1 h after drug administration (Fig. 1). This may suggest a hypothalamic site of action of GABA on the pituitary-adrenocortical activity. When given systematically GABA induced a biphasic effect. In lower doses (1—50 mg/kg) it consistently decreased the resting serum corticosterone levels, statistically significantly after a dose of 10 mg/kg, and in larger doses, 100—500 mg/kg, it considerably raised the corticosterone concentration in blood serum (Fig. 1).

Muscimol administered icv, (0.01—0.5 μg) induced a most potent and significant increase in serum corticosterone levels when given in a dose of 0.1 μg. Given systemically in a dose of 1 mg/kg, muscimol also induced a significant rise in corticosterone levels in blood serum (Fig. 2).

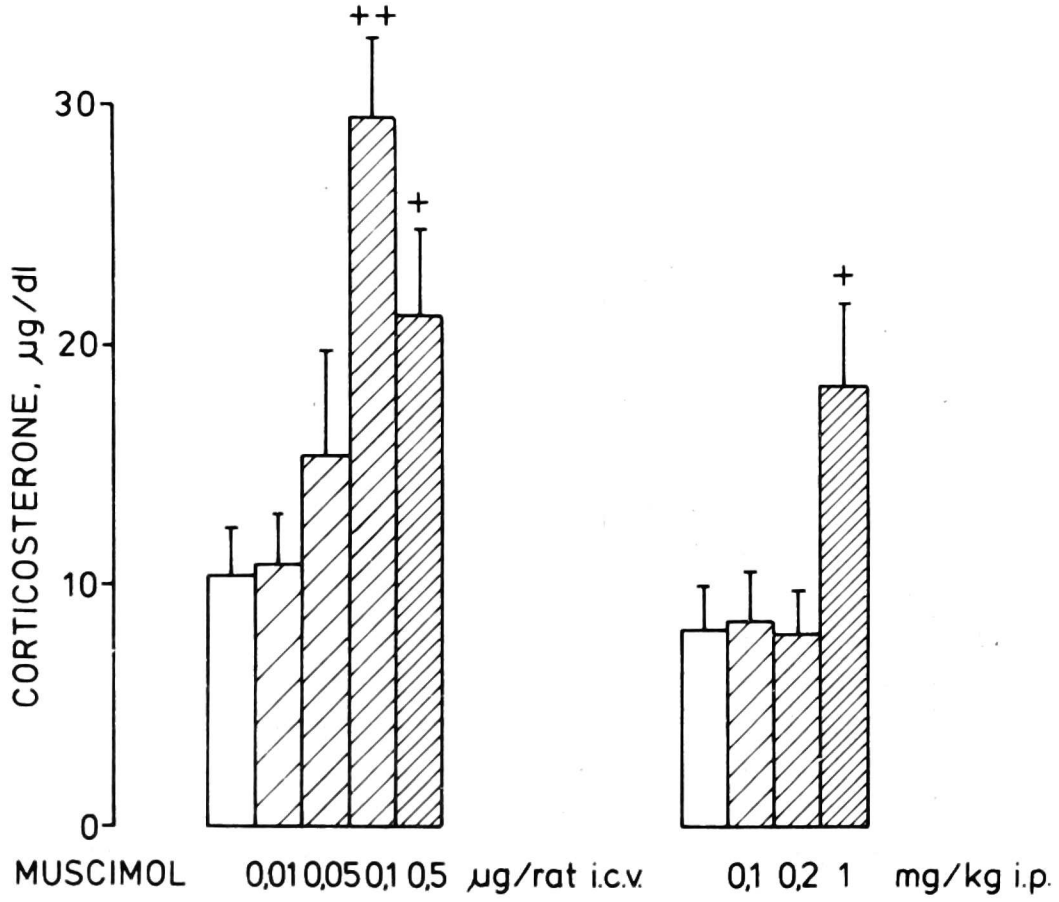

Fig. 2. Serum corticosterone concentrations in conscious rats 1 h after icv and ip muscimol administration. Bars represent means, vertical lines SEM of 7—14 animals in each group. + $p<0.05$ and ++ $p<0.001$ vs. saline-treated group.

Effect of bicuculline

Under condition of present experiment bicuculline did not affect serum corticosterone levels. Administered intraventricularly (0.01—1 μg) or systemically (0.01—2 mg/kg) bicuculline did not significantly influence the resting serum corticosterone levels as compared with the levels in respective saline treated controls. Also the differences between any pair of corticosterone values after different doses of bicuculline, separately for icv and ip administration, were not statistically significant (Table 1).

Tab. 1. Effect of bicuculline on serum corticosterone levels in rats.

Treatment	Dose	Corticosterone μg/dl
Saline control	10 μl icv	8,4 ± 1,6
Bicuculline	0,001 μg icv	8,6 ± 1,9
Saline control	10 μl icv	9,1 ± 1,6
Bicuculline	0,01 μg icv	12,6 ± 3,3
Saline control	10 μl icv	6,4 ± 0,9
Bicuculline	0,1 μg icv	5,8 ± 0,7
Saline control	10 μl icv	8,4 ± 1,6
Bicuculline	1 μg icv	9,7 ± 0,4
Saline control	0,2 ml ip	8,4 ± 1,0
Bicuculline	0,01 mg ip	7,4 ± 1,8
Saline control	0,2 ml ip	7,3 ± 1,3
Bicuculline	0,1 mg ip	9,0 ± 2,1
Saline control	0,2 ml ip	8,2 ± 1,5
Bicuculline	2 mg ip	8,3 ± 2,2

Bicuculline was injected icv or ip and 1 h later the rats were decapitated. Each value represents the mean ± SEM of 7—14 rats.

Effect of bicuculline on the GABA-induced corticosterone responses

Bicuculline administered icv (0.001—0.1 μg) 15 min prior to GABA given by the same route, totally abolished the rise in serum corticosterone response induced by GABA. This may suggest that GABA stimulates the CRF containing neurons within the hypothalamus.

A significant rise in the serum corticosterone levels induced by GABA (500 mg/kg) given ip was not affected by ip preteatment 15 min earlier with bicuculline 0.01—0.1 mg/kg, and pretreatment with larger dose of bicuculline, 2 mg/kg, intensified the effect of GABA on corticosterone secretion (Fig. 3).

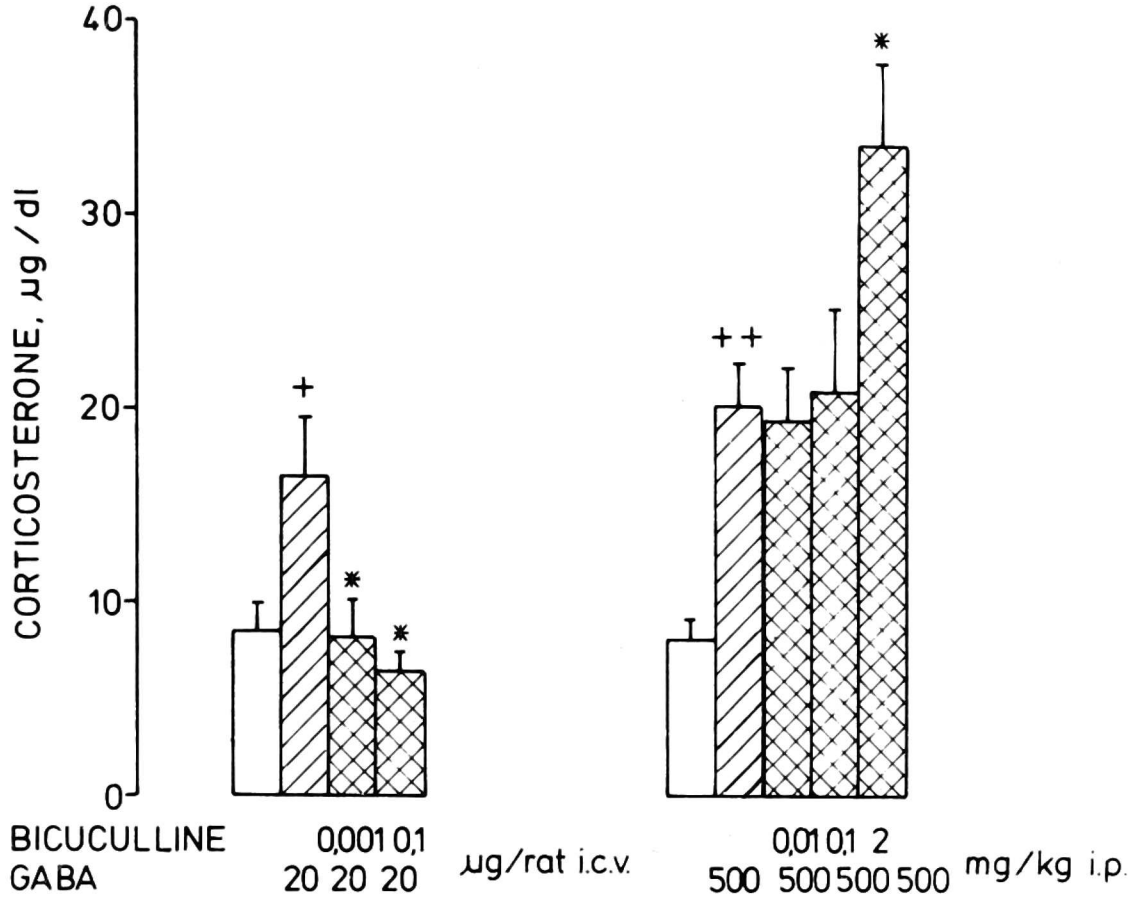

Fig. 3. Effect of bicuculline on GABA-induced corticosterone concentrations. Bicuculline was given 15 min before GABA. Bars represent means, vertical lines SEM of 7—14 animals in each group. + $p<0.05$ and + + $p<0.001$ vs. saline-treated group; * $p<0.05$ vs. GABA-treated group.

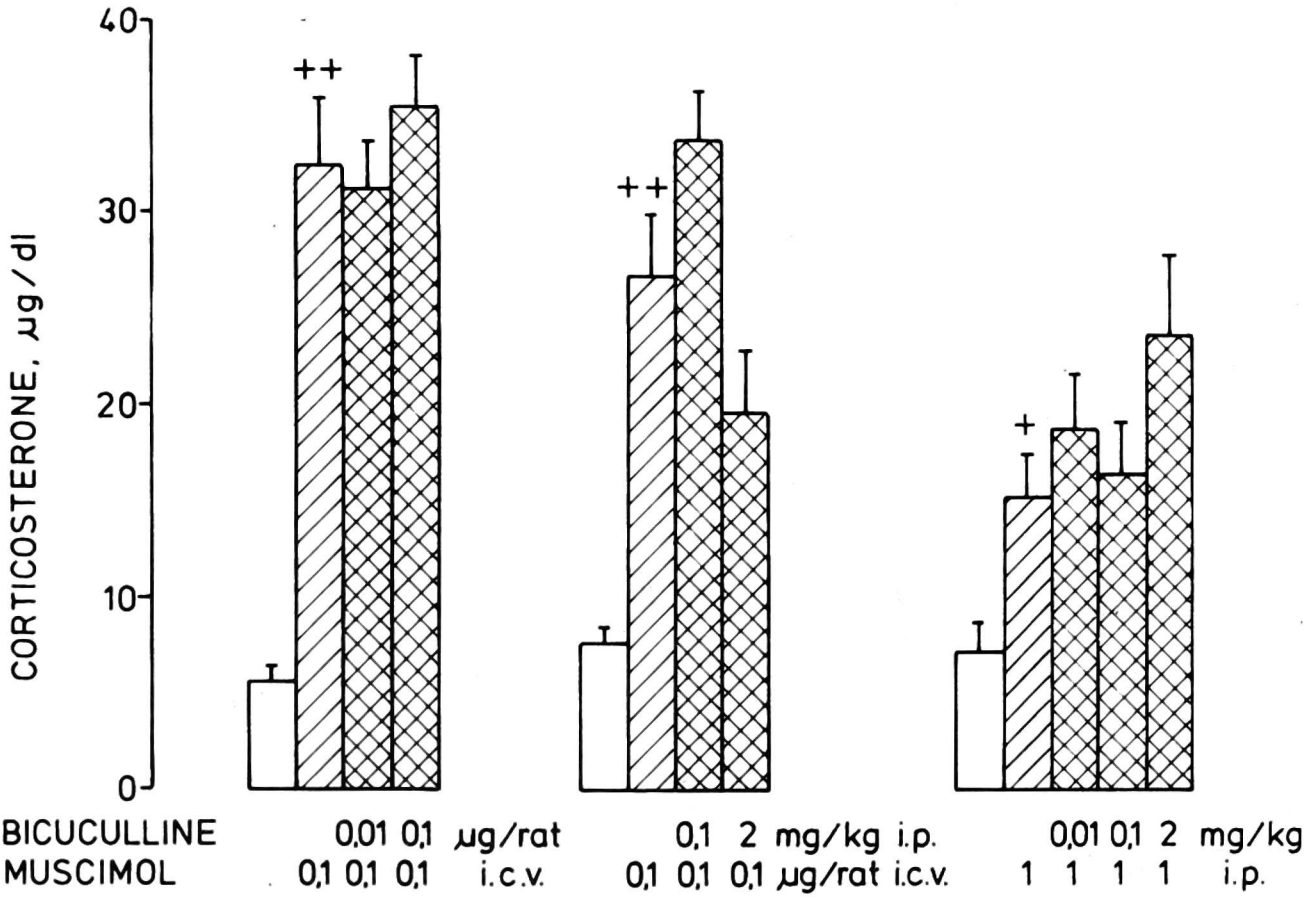

Fig. 4. Effect of bicuculline on muscimol-induced corticosterone concentrations. Bicuculline was given 15 min before muscimol. Bars represent means, vertical lines SEM of 4—14 animals in each group. + $p<0.05$ and + + $p<0.001$ vs. saline treated controls.

Effect of bicuculline on muscimol-induced corticosterone response

Intraventricular pretreatment of rats with bicuculline (0.01—0.1 μg) did not significantly affect the increase in serum corticosterone levels induced by muscimol given by the same route. This result is in contrast with the total inhibition by the same doses of bicuculline of the GABA-induced corticosterone responses. Also systemically injected bicuculline (0.01—2 mg/kg) did not significantly antagonize the hormone response induced by either icv or ip administered muscimol (Fig. 4).

Effect of atropine on corticosterone responses to GABA and muscimol

Atropine (0.001—0.1 μg) injected icv in doses which themselves did not influence the resting serum corticosterone levels left unchaged the increase in corticosterone concentration induced by GABA, given by the same route. On the contrary, the same doses of atropine significantly diminished (by 33%) the increase in serum corticosterone induced by a subsequent icv administration of muscimol (Fig. 5). This suggests a partial involvement of central cholinergic, muscarinic receptors in stimulation of the pituitary-adrenocortical activity exerted at the hypothalamic level.

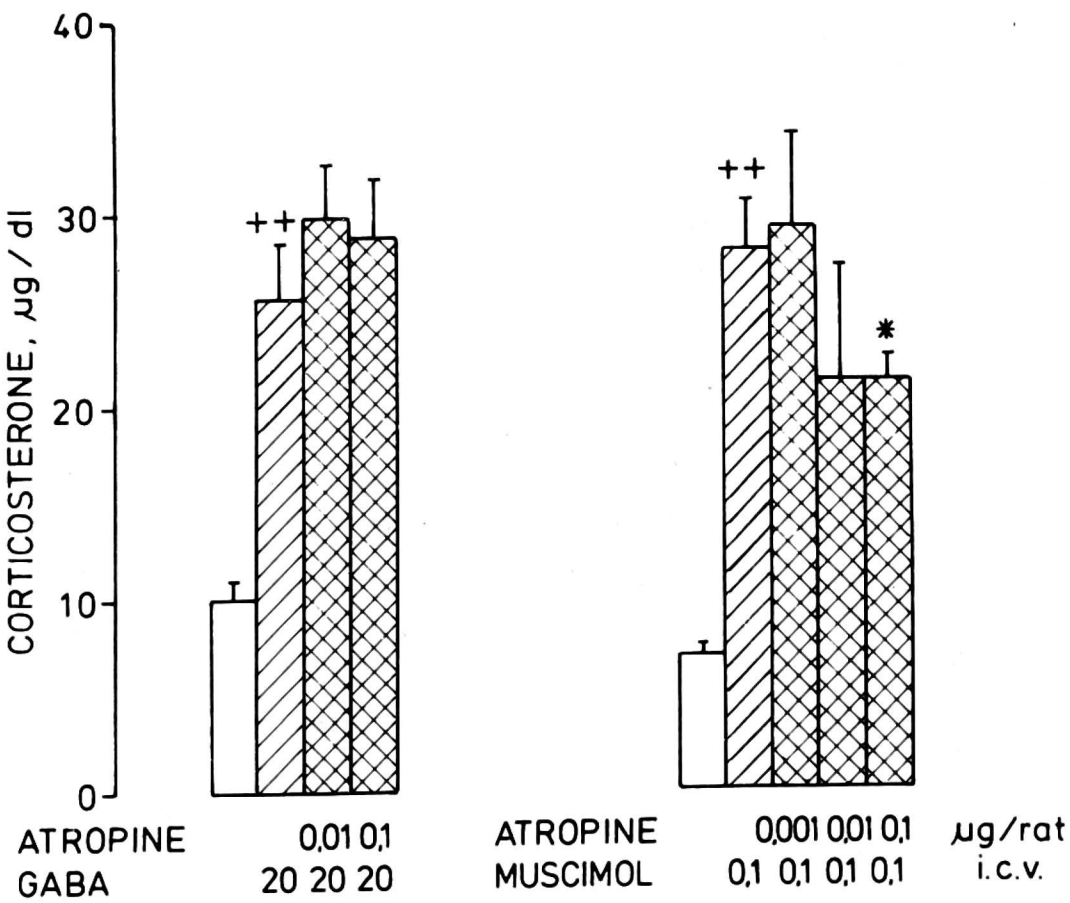

Fig. 5. Effect of atropine on GABA- and muscimol-induced corticosterone responses. Atropine was given 15 min before GABA and muscimol. Bars represent means, vertical lines SEM of 7—14 animals in each group. ++ $p<0.001$ vs. saline-treated group, * $p<0.05$ vs. muscimol-treated group.

DISCUSSION

In order to elucidate the role and site of GABA receptors involved in control of the hypothalamic-pituitary-adrenal axis activity the GABA-ergic agonists and antagonist were injected intraventricularly and/or intraperitoneally. It is known that GABA and bicuculline do not penetrate easily the blood-brain barrier from systemic circulation and depending on a route of administration they act either at the pituitary or hypothalamic level.

In the present experiments GABA given icv significantly and dose-dependently raised the serum corticosterone levels. This increase was totally suppressed by icv pretreatment with bicuculline a specific antagonist of $GABA_A$-receptors. This suggests that stimulation of these receptors within the hypothalamus (4), possibly at the neurosecretory hypophysiotropic cells (13, 16), is responsible for the activation by GABA of the pituitary-adrenal axis. This assumption is confirmed by the fact that glutamic acid decarboxylase, the GABA-synthesizing enzyme, has been found in some corticotropin-releasing-factor-containing neurons of the paraventricular nucleus (16). In contrast to our results Miguez et al. (17, 18) found that GABA infused into the lateral ventricle of conscious rats induced a marked decrease in the corticosterone levels which would suggest an inhibitory effect on ACTH release. Although these authors claimed the rats as non stressed, their resting corticosterone levels exceeded 20 μg/dl which is evidently stress level, and GABA was reported to inhibit the pituitary-adrenocortical response to stressful condition (13, 19).

In the present experiment GABA given systemically induced a biphasic effect on the corticosterone secretion. In a small dose, 10 mg/kg ip, it repeatedly and significantly decreased the resting serum corticosterone level whereas in larger doses, 100—500 mg/kg, it considerably raised the serum hormone concentration. Since GABA does not easily cross the blood-brain barrier, the inhibitory effect of systemically injected GABA in smaller doses could be due to a direct effect on the pituitary corticotrophs (20—22), like a similar inhibitory effect on prolactin secretion (10, 11, 23). The increase in serum corticosterone levels observed in the present experiment after larger doses of GABA given systemically could be due to a partial penetration of the hypothalamus where GABA exerts a stimulatory effect on CRF/ACTH secretion (24, 25). The present results suggest the existence of two distinct componenets of GABA action on ACTH/corticosterone secretion. It appears that pituitary GABA receptors play a major role in the inhibition of ACTH/corticosterone release induced by peripheral administration of GABA, whereas hypothalamic GABA receptors are involved in stimulation of CRF release. After intraperitoneal administration of larger doses of GABA an increase in both serum corticosterone and hypothalamic noradrenaline levels

were observed (12) and noradrenaline given icv is known to stimulate the hypothalamic-pituitary-adrenocortical activity in rats (26).

In the present experiment muscimol, a $GABA_A$-receptor agonist, given in effective doses increased the serum corticosterone levels after both intaventricular and intraperitoneal administration. We did not observe any biphasic effect of muscimol injected ip on corticosterone secretion. Muscimol is known to stimulate ACTH and corticosterone and prolactin secretion after both systemic and central administration (11, 27, 28). Since the concentration of muscimol in different brain areas after central administration was similar to that after systemic injection (11), both pituitary and hypothalamic receptors may be involved in its stimulatory effect. In the present experiment intraventricular pretreatment with bicuculline, in doses which completely antagonized the GABA-induced corticosterone secretion, did not alter the hormone responses evoked by a subsequent icv administration of muscimol. Also given ip bicuculline did not antagonize but slightly increased the corticosterone responses induced by both icv and ip administration of muscimol. These results are difficult to interpret since both muscimol and bicuculline are considered to be specific GABA-ergic agents. The pituitary-adrenocortical stimulation induced by muscimol could be due to release of neurotransmitters by activation of bicuculline-intensitive GABA receptors (25) and bicuculline is known to posses some effects unrelated to $GABA_A$-receptor blockade (29).

Our results indicate that a significant part of muscimol-induced corticosterone response depends on central muscarinic receptors, since icv pretreatment with atropine considerably impaired the increase in corticosterone secretion induced by a subsequent administration of muscimol. By contrast, atropine did not change the corticosterone secretion induced by GABA. The mechanism by which muscimol increases the pituitary-adrenocortical activity needs further investigation. The present results indicate that both GABA and muscimol stimulate the pituitary-adrenal activity at the hypothalamic level, and stimulation by GABA, but not muscimol, is entirely mediated by $GABA_A$-receptors, whereas in the stimulation by muscimol hypothalamic cholinergic, muscarinic receptors are significantly involved.

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