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Progabide, A GABA Mimetic Drug, Stimulates the Secretion of Plasma Corticosterone in Rats

HARI MANEV AND DANKA PERIČIĆ

Department of Experimental Biology and Medicine, "Rudjer Bošković" Institute Bijenička 54, 41000 Zagreb, Yugoslavia

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MANEV, H. AND D. PERIČIĆ. *Progabide, a GABA mimetic drug, stimulates the secretion of plasma corticosterone in rats.* PHARMACOL BIOCHEM BEHAV **28**(4) 443–446, 1987.—The gamma-aminobutyric acid (GABA) receptor agonist, progabide, was administered intraperitoneally to male Wistar rats. Doses of 50, 100 and 200 mg/kg increased the plasma corticosterone levels by 244, 365 and 476% respectively. Ten mg/kg was ineffective. The enhancement of plasma corticosterone level induced by 200 mg/kg of progabide lasted for 2 hours. The pretreatment of rats with the synthetic corticoid dexamethasone (3 days, 1 mg/kg daily) lowered the plasma corticosterone concentration and abolished its rise induced by progabide. Alpha-2 adrenergic agonist, clonidine (1 mg/kg), elevated the resting plasma corticosterone level but diminished the progabide-induced increase of plasma corticosterone concentration. GABA-A receptor blocking agent, picrotoxin (3 mg/kg), as well as ether stress increased the basal corticosterone level, but both treatments were without effect in progabide-pretreated rats. The results suggest that progabide stimulates the secretion of corticosterone by acting at a site different than the adrenal cortex. It appears that GABA-agonistic activity of progabide is not directly responsible for this effect.

Progabide GABA agonist Plasma corticosterone Rat Picrotoxin Ether stress Clonidine Dexamethasone

PROGABIDE (SL 76002; [[(4-chlorophenyl)(5-fluoro-2-hydroxyphenyl)-methylene]amino]butanamide), a specific gamma-aminobutyric acid (GABA) receptor agonist [16], has recently been extensively investigated, and its usefulness in the treatment of some neurologic and psychiatric disorders has been proposed (see [4,5]). However, the data on the neuroendocrine effects of progabide are scarce [17].

Recent results from both morphological and functional studies have supported the presumption that the inhibitory neurotransmitter GABA may have a neuroendocrine function. The immunohistochemical techniques [30] and radioligand binding studies [2] have revealed the presence of elements of GABA neuron system in the hypothalamus and the pituitary gland (see also [27]). Factors such as stress, which activate the hypothalamo-pituitary-adrenal (HPA) axis and elicit an increased secretion of corticosteroids, also affect the GABA system located in this axis [19,21].

On the other hand, pharmacological manipulations of GABAergic transmission induce profound changes in the activity of the HPA axis [3, 18, 20, 23, 26, 28]. Although it has been suggested that GABA, generally speaking, inhibits the activity of the HPA axis [12], there are still contradictory data on the influence of the particular GABAergic drug on the secretion of plasma corticosterone. This might, in part, be attributed to differences in the specificity of GABAergic drugs used, as well as to diversity of mechanisms by which various GABAergic drugs affect the GABA system. Unfortunately, the number of selective GABA agonistic drugs is very limited. Hence, the aim of the present study was to investigate the influence of progabide, a drug supposed to have a specific GABA agonistic activity, on the secretion of plasma corticosterone in rats.

METHOD

Male Wistar rats (of our institute) weighing 170–240 g were caged in groups of three under diurnal lighting conditions with free access to food and water. The rats were accustomed to handling (housing 1 per cage for 30 min, injecting with saline, 1 ml/100 g body weight) in the period of 7 days before the beginning of the experiment.

Progabide (SL 76002; L.E.R.S. Synthélabo, Paris, France) was injected as a suspension in saline containing 1‰ Tween 80. Picrotoxin (Sigma Chemical Co., St. Louis, MO), clonidine HCl (Catapresan; Boehringer, Ingelheim on Rhein, FRG) and dexamethasone (Krka, Novo Mesto, Yugoslavia) were dissolved in saline. All drugs were given intraperitoneally (IP). Control rats were treated IP with the corresponding vehicle (1 ml/100 g body weight). Ether stress was performed as described previously [19,21]. Briefly, animals were placed for 2 min in the glass jar containing ether vapour, then they were removed and after 11 min placed again for 2 min in the ether containing jar. At the end of the second exposure to the ether, i.e., 15 min after the beginning of the stressful procedure, the animals were sacrificed.

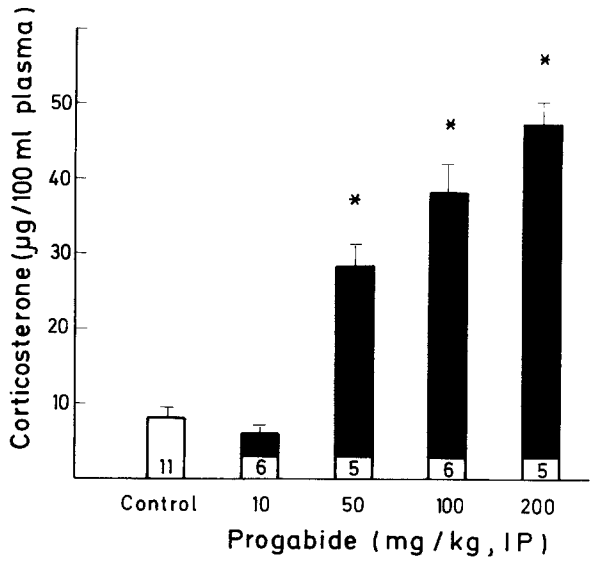

FIG. 1. Effect of progabide on plasma corticosterone levels: dose response. Progabide or its vehicle (1‰ Tween 80 in saline) were administered IP 60 min prior to death. Bars represent mean±SEM. Numbers in the bars represent number of animals in the group. *$p<0.01$ when compared with the control, vehicle-treated group (ANOVA followed by Dunnett's t-test).

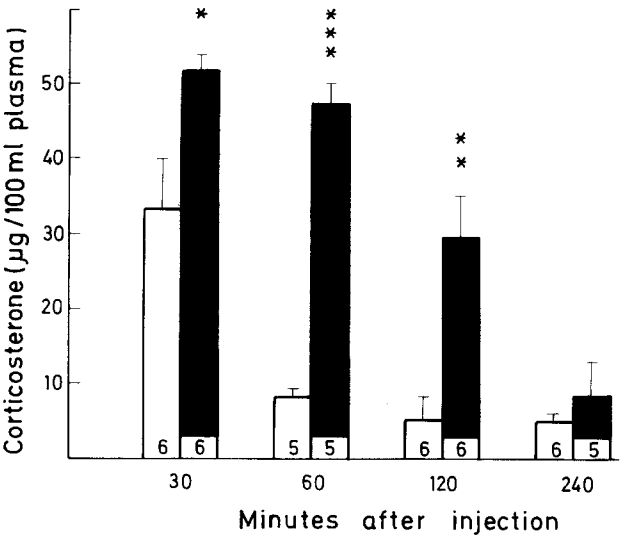

FIG. 2. Effect of progabide on plasma corticosterone levels: time course. Progabide (200 mg/kg) or its vehicle were administered IP 30, 60, 120 and 240 min prior to death. Bars represent mean±SEM. Numbers in the bars represent number of animals in the group. Columns: open, vehicle-treated controls; dark, groups treated with progabide. *$p<0.05$, **$p<0.01$, ***$p<0.001$ when comprared with the corresponding vehicle-treated control (Student's t-test).

TABLE 1

EFFECT OF PROGABIDE ON PLASMA CORTICOSTERONE LEVELS IN MALE RATS PRETREATED WITH DEXAMETHASONE

Treatment	Plasma Corticosterone (µg/100 ml)	(n)
Control (saline + vehicle)	12.45 ± 3.24	(5)
Dexamethasone + vehicle	4.27 ± 0.49*	(5)
Dexamethasone + progabide	4.03 ± 0.29†	(6)

Dexamethasone (1 mg/kg) or saline were administered IP for 3 consecutive days. The last dose of dexamethasone was given 12 hours prior to death. Progabide (200 mg/kg) or its vehicle were injected IP 60 min prior to death. Data are expressed as the mean ± SEM of (n) animals in the group.

*$p<0.05$, †$p<0.025$ when compared with the control group (ANOVA followed by Scheffe's test).

The animals were killed by decapitation with a guillotine. Trunk blood obtained after decapitation was collected in heparinized beakers, centrifuged, and plasma was stored at −20°C. To avoid the influence of the circadian rhythm on plasma corticosterone concentration all experiments were carried out between 09.00 and 13.00. Plasma corticosterone levels were determined by the fluorimetric method [22,29].

Statistical analysis of the results was performed by two-tailed Student's t-test and by analysis of variance (ANOVA) followed by Dunnett's (multiple comparison with a control) or Scheffe's (multiple comparison) procedure. The criterion for significance in all tests was $p<0.05$.

RESULTS

Doses of 50, 100 and 200 mg/kg progabide given IP to male Wistar rats increased the plasma corticosterone levels by 244, 365 and 476% respectively ($t=6.44$–12.55, $p<0.01$, Dunnett's test). Ten mg/kg of progabide failed to change the concentration of corticosterone in plasma ($t=0.76$, N.S.) (Fig. 1).

The increased plasma corticosterone level induced by administration of 200 mg/kg progabide was evident 30 ($t=2.625$, $p<0.05$), 60 ($t=13.13$, $p<0.001$) and 120 min ($t=4.07$, $p<0.01$) after drug injection, but returned to the control value 240 min after drug injection ($t=0.86$, N.S., Student's test) (Fig. 2). Figure 2 also shows that the greatest corticosterone rise (compared with the corresponding control) was reached 60 min following the administration of progabide. (The peak of the absolute value of plasma corticosterone was observed 30 min following progabide.)

Dexamethasone treatment (1 mg/kg; administered IP for 3 consecutive days) decreased significantly the plasma corticosterone concentration, $F(2,13)=9.07$, $p<0.05$, and, as indicated by Scheffe's test, prevented the progabide-induced elevation of corticosterone level, $F(2,13)=10.48$, $p<0.025$, when compared with the control group; $F(2,13)=0.01$, N.S., when compared with dexamethasone treated group (Table 1).

Table 2 shows that the GABA blocking agent picrotoxin (3 mg/kg) and the ether stress (2×2 min within 15 min) stimulate the secretion of plasma corticosterone in basal conditions ($t=9.49$ and 10.90 respectively, $p<0.001$, Student's test), but not in progabide (200 mg/kg) pretreated rats. On the other hand, clonidine (1 mg/kg), which also stimulated the secretion of corticosterone in basal conditions ($t=8.96$, $p<0.001$), diminished it by 33% in progabide-pretreated rats ($t=5.33$, $p<0.001$, Student's test) (Table 2).

TABLE 2

EFFECT OF PICROTOXIN, ETHER STRESS OR CLONIDINE ON THE BASAL AND PROGABIDE-ELEVATED PLASMA CORTICOSTERONE LEVELS IN RATS

Plasma corticosterone			
Basal Conditions (% of saline-treated control)		Progabide Pretreatment (% of progabide-treated control)	
Saline	100.00 ± 23.27 (6)	Progabide	100.00 ± 4.08 (6)
Picrotoxin	431.61 ± 26.07* (6)	Progabide + picrotoxin	107.34 ± 6.75 (6)
Saline	100.00 ± 26.11 (6)	Progabide	100.00 ± 5.27 (6)
Ether stress	436.18 ± 16.49* (6)	Progabide + ether stress	99.97 ± 6.81 (6)
Saline	100.00 ± 10.12 (5)	Progabide	100.00 ± 4.08 (6)
Clonidine	257.65 ± 14.40* (6)	Progabide + clonidine	67.18 ± 4.61* (6)

Picrotoxin (3 mg/kg), clonidine (1 mg/kg) or saline (1 ml/100 g) were administered IP 60 min prior to decapitation. Progabide (200 mg/kg) was administered IP 65 min prior to death. Rats exposed to ether stress (2×2 min within 15 min) 45 min following IP saline (basal conditions) or 50 min following progabide (progabide pretreatment). The results (mean ± SEM) are expressed as % of the corresponding control. Numbers in parentheses represent number of animals in the group.

*$p<0.001$ when compared with the corresponding control (Student's t-test).

The rise of plasma corticosterone induced by progabide pretreatment was not affected by administration of drugs which act on dopaminergic (apomorphine: 3 mg/kg), cholinergic (physostigmine: 1 mg/kg; atropine: 10 mg/kg) or serotoninergic (parachlorophenylalanine: 2×150 mg/kg, i.e., 48 and 24 hr prior to progabide) system (data not shown).

DISCUSSION

Our present data show the stimulatory influence of a GABA agonistic drug progabide on the secretion of corticosterone, what might be in line with some rare findings on the stimulatory influence of GABA on the activity of the HPA axis [1,10]. This effect of progabide was most prominent 60 min after its administration (compared with the corresponding control animals), although the greatest absolute value of plasma corticosterone was achieved 30 min following drug administration. Namely, due to stressful action of IP injection, 30 min following vehicle administration plasma corticosterone levels were still elevated in the control group (compared with values observed 60, 120 and 240 min after the injection of vehicle). (This also has been observed in our earlier studies; see [23].)

Although one might speculate that our present data suggest that progabide is one of the many stressful substances which may stimulate corticosterone secretion if given in high enough doses, it should be emphasized that in our experimental conditions progabide stimulated corticosterone secretion starting from 50 mg/kg, and most of the pharmacological (both biochemical and behavioural) properties of this drug have been observed following much higher dosage (see [4,5]).

According to our recent findings one might presume that the effect of progabide would be different if female instead of male rats have been used. Namely, we have recently reported that the sex of animals influences the plasma corticosterone response to GABA-related drugs [24]. However, the administration of progabide to female rats also produces only elevations of plasma corticosterone levels [25].

To exclude the possibility of a direct stimulatory effect of progabide on the adrenal cortex we pretreated rats with the synthetic corticoid dexamethasone. As expected, dexamethasone lowered the plasma corticosterone concentration (Table 1), but it also prevented the progabide-induced rise of plasma corticosterone. Accordingly, it might be concluded that the effect of progabide, observed in the present study, has resulted from the action of the drug at the level superior to adrenal cortex, presumably at the hypothalamo-pituitary level.

The bulk of evidence from either in vivo or in vitro studies suggests the inhibitory influence of GABA on the HPA axis, at least when the activated HPA axis is being studied [3, 8, 9, 13, 18, 19, 26]. The activation of the HPA axis by ether stress has been shown to affect the hypothalamic GABA system [19]. On the other hand, administration of GABA potentiating drugs, benzodiazepines, was able to diminish or prevent the stress-induced elevation of plasma corticosterone in spite of the stimulating influence of these drugs on the resting corticosterone levels [6,19]. Since in our study ether stress could not elevate plasma corticosterone levels in progabide-pretreated rats (Table 2) one might presume that progabide prevented the effect of ether stress on the HPA axis.

However, having in mind that the elevation of corticosterone levels following progabide administration was the greatest elevation ever observed in our experiments (see Figs. 1, 2) one might presume that progabide provoked the maximal stimulation of the adrenal cortex, and that no further increases of plasma corticosterone were possible. This might explain the failure of both ether stress and picrotoxin (which stimulated the basal secretion of corticosterone) to affect the plasma corticosterone levels in progabide-pretreated rats (Table 2).

Since picrotoxin, a drug which blocks GABA-A receptor complex (see [7]), increased plasma corticosterone levels it has often been concluded that GABA inhibits the activity of the HPA axis [11, 18, 20], and we have suggested that the stimulatory effect of picrotoxin on the HPA axis is mediated via GABA-A receptors [14]. From the fact that progabide, a mixed GABA-A/GABA-B receptor agonist produces the same final effect as picrotoxin, it might be presumed that the stimulatory effect of progabide on the secretion of corticosterone is not mediated via GABAergic mechanisms, or at least not via those which include picrotoxin-sensitive GABA-A receptor sites.

The involvement of noradrenergic system has been implied in the stimulatory influence of GABA [1] and diazepam [14] on the activity of the HPA axis. As shown in Table 2, the alpha-2 adrenergic agonist, clonidine [15], stimulated the secretion of corticosterone in basal conditions, but diminished it in progabide-pretreated rats. On the other hand, the

progabide-induced rise of plasma corticosterone was not affected by administration of drugs which act on dopaminergic, cholinergic or serotoninergic systems (data not shown). Accordingly, it appears that the interaction of progabide or progabide-induced changes in GABA system with the noradrenergic system might be involved in the progabide-induced elevation of plasma corticosterone levels. Similar to our findings are the results of Boaventura *et al.* [6], who have reported the stimulatory effect of clonidine (0.05–10.0 mg/kg, IP) on the secretion of plasma corticosterone in rat, and who also have found that in combination with benzodiazepines (which by themselves stimulated the secretion of corticosterone) the plasma corticosterone levels may be diminished.

In conclusion, the present study shows the stimulatory influence of a mixed GABA-A/GABA-B receptor agonist progabide on the plasma corticosterone secretion in male rats. This effect of the drug is not achieved by its direct action on the adrenal cortex, and does not appear to be mediated via GABA-A receptors. The exact mechanism by which progabide elevates plasma corticosterone should be further studied.

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