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Hypothalamic GABA System and Plasma Corticosterone in Ether Stressed Rats

HARI MANEV AND DANKA PERIČIĆ[1]

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

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MANEV, H. AND D. PERIČIĆ. *Hypothalamic GABA system and plasma corticosterone in ether stressed rats.* PHARMACOL BIOCHEM BEHAV **18**(6) 847–850, 1983.—Exposure of rats to the ether stress (2×2 min within 15 min) activated the hypothalamo-hypophyseal-adrenal (HHA) axis, as evidenced by the increased plasma corticosterone concentration, and affected the hypothalamic GABA system. The aminooxyacetic acid (AOAA) or L-cycloserine-induced accumulation of GABA was decreased, and the activity of glutamate decarboxylase (GAD), the enzyme responsible for GABA synthesis, was increased following ether stress. The concentration of GABA and the activity of GABA: 2-oxoglutarate aminotransferase (GABA-T), the enzyme responsible for GABA catabolism, remained unchanged under given conditions. Diazepam, a drug known to potentiate GABA-ergic transmission, elevated the concentration of plasma corticosterone, but prevented its further increase by ether stress. In spite of the diminished accumulation of GABA, the results might suggest that ether stress activates the hypothalamic GABA system, which is then able to prevent a further response of the HHA axis to stress.

Ether stress Hypothalamus GABA GAD GABA-T Plasma corticosterone Diazepam

ENVIRONMENTAL stressors affect the brain neurochemical systems and the endocrine systems producing a new interplay between them. It is generally assumed that stress activates the hypothalamo-hypophyseal-adrenal (HHA) axis. Numerous investigations have been made to elucidate the involvement of different neurotransmitters in the control of the HHA axis activity. The same attempts have been made for GABA, the inhibitory neurotransmitter of the central nervous system.

The hypothalamus contains a relatively high amount of GABA [20], which is supposed to be located in short interneurons [33], but it also can be carried from the median eminence through the hypophyseal portal vessels to reach specific receptor sites located in the anterior pituitary [30]. GABA has been reported to stimulate [9], to inhibit [2, 18, 24] and not to affect [1,8] the HHA activity. An easily measurable indicator of the HHA activity is the concentration of corticosterone in plasma. Both plasma corticosterone [3] and the hypothalamic GABA [4] show circadian fluctuations which appear to be in a negative correlation. There are data which might suggest that such a correlation also might exist under the stressful conditions [17,24]. However, there are relatively few reports showing the effect of stress on the hypothalamic GABA system [10,14], and to our knowledge in only one of them has the concentration of plasma corticosterone been followed in parallel [14]. With aim to further elucidate the interaction between the HHA axis and the hypothalamic GABA system under stressful conditions we studied in parallel: (1) changes in the plasma corticosterone levels and the activity of hypothalamic GABA system in rats exposed to the acute ether stress; (2) changes in the plasma corticosterone levels of the ether stressed rats which have been pretreated with diazepam, a drug known to potentiate the function of the GABA system. Ether was chosen as a stressful stimulus because of its ability to elicit very intensive corticotropin (ACTH) and corticosterone secretion in the rat, even after very short action [6].

METHOD

Male Wistar rats, weighing 140–200 g were caged in groups of three under diurnal lighting conditions. They were given food and water ad lib. The rats were accustomed to handling in the period of seven days before the beginning of the experiments.

The experimental animals were placed for two minutes in the glass jar containing ether vapour, then they were removed and after eleven minutes placed again for two minutes in the ether containing jar. At the end of the second exposure to the ether, i.e., 15 min after the beginning of the procedure, the animals were killed by decapitation with a guillotine in the experiments where enzyme activity and plasma corticosterone concentration were determined, or by exposing their heads to a focussed beam of microwave irradiation for 5.0 sec [13] in experiments in which GABA levels were measured. Unstressed rats were undisturbed and sacrificed immediately after removal from the cage. To avoid the influence of the circadian rhythm on the hypothalamic GABA level and plasma corticosterone concentration, all experiments were carried out between 9:00 a.m. and 13:00 p.m.

[1]Requests for reprints should be addressed to Danka Peričić, "Rudjer Bošković" Institute, Bijenička cesta 54, 41000 Zagreb, P.O.B. 1016, Yugoslavia.

Aminooxyacetic acid hemihydrochloride (AOAA, Eastman Kodak Co., Rochester, NY) was administered intraperitoneally (IP) 70 min before death. L-cycloserine (F. Hoffmann la Roche and Co. A. G., Basel, Switzerland) and diazepam (Apaurin, Krka, Novo Mesto, Yugoslavia) were administered IP 180 and 70 min respectively before death. Adrenocorticotrophin (ACTH, Galenika, Beograd, Yugoslavia) was given 15 min before death. Control animals were treated IP with saline 1 ml/100 g of body weight.

After sacrifice the brains were rapidly removed from the skulls and placed in a tissue slicer over an ice cold plate. A tissue slicer with a brain shaped depression 22 mm long, and slits at 1.5 mm intervals, was used to obtain the region of the hypothalamus. Hypothalamic punch, 3 mm in diameter, was taken from the area, which, looking from the anterior surface of the slice, lies between commissura anterior and chiasma opticum [19]. This punch contained predominantly the tissue of the medial hypothalamus, the brain region thought to be responsible for the increase of corticosterone following ether stress [11,27]. The nuclei which according to Tappaz *et al.* have the highest activity of GAD, i.e., preoptic, anterior and dorsomedial nuclei, were included in the punch [34]. GABA was determined by a modification [29] of the enzymatic fluorimetric method of Hirsh and Robins [16]. GAD activity was measured, in the presence and absence of 0.1 mM pyridoxal phosphate, during a 5 min incubation period using 5 mM concentration of L-[U-^{14}C] glutamic acid [25]. GABA:2-oxoglutarate aminotransferase (GABA-T) activity was determined by a modification [36] of the method of Hall and Kravitz [15]. Protein concentrations were determined in 10 μl of the homogenates according to Lowry *et al.* [23].

Trunk blood, obtained after decapitation, was collected in heparinized beakers, centrifuged, and plasma was stored at −20°C. Plasma corticosterone level was determined by a slight modification of the fluorimetric methods [26,31].

Statistical analysis of the results was performed by two tailed Student's *t*-test and by the analysis of variance followed by Scheffe's multiple comparison procedure. The criterion for significance in both tests was $p<0.05$.

RESULTS

Exposure of rats to the ether stress (2×2 min) significantly elevated ($p<0.001$ by 178%) the plasma corticosterone concentration (Table 3), but failed to change GABA level in the hypothalamus (Table 1). Since the measurement of neurotransmitter concentration does not necessarily reflect the activity of the corresponding neurons, we also studied the influence of ether stress on the synthesis of GABA. It has been suggested that following the accumulation of GABA after treatment with the inhibitor of GABA catabolism, AOAA, may provide a simple means of screening for *in vivo* drug induced alterations in GABA synthesis [28]. The administration of AOAA (25 mg/kg) as well as the administration of another inhibitor of GABA-T, L-cycloserine (50 mg/kg), increased the hypothalamic GABA level. This increase was more pronounced following L-cycloserine administration. Ether stress significantly ($p<0.05$) diminished both the AOAA and L-cycloserine induced GABA accumulation in the hypothalamus (Table 1). As shown in Table 2 *in vitro* measured activity of GAD, the enzyme responsible for GABA synthesis, showed a modest (10%) but significant ($p<0.01$) increase in the hypothalami of ether stressed rats, both in the absence and in the presence of added pyridoxal phosphate. On the other hand, ether stress did not produce any change in the activity of hypothalamic GABA-T (Table 2). Both enzymes require pyridoxal phosphate as cofactor. In order to find out whether ether stress affects the saturation of both enzymes with cofactor, we calculated these saturations. Table 2 shows that GABA-T is almost completely (96%) and GAD 71% saturated by cofactor, and that *in vitro* measured saturation with cofactor of neither GAD, nor GABA-T is affected by previous exposure of the animals to the ether stress.

TABLE 1

EFFECT OF ETHER STRESS ON THE AMINOOXYACETIC ACID (AOAA)- AND L-CYCLOSERINE-INDUCED GABA ACCUMULATION IN THE HYPOTHALAMUS

	GABA (nmoles/mg protein)	
	Control	Ether stress
Untreated	38.15 ± 1.46 (10)	38.15 ± 1.65 (12)
AOAA	46.21 ± 1.55 (12)	41.94 ± 1.16* (11)
L-Cycloserine	58.35 ± 2.33 (5)	50.97 ± 1.36* (5)

AOAA (25 mg/kg) or L-cycloserine (50 mg/kg) were administered IP 70 and 180 min respectively prior to death. Rats were killed immediately after ether stress (2×2 min within 15 min). The results are the mean ± SEM. Numbers in parentheses represent number of experiments.

*$p<0.05$ when compared with the corresponding control group.

Treatment with 10 mg/kg of diazepam elevated by 185% the resting level of plasma corticosterone, $F(5,37)=114.29$, $p<0.001$, but the exposure of so treated rats to the ether stress failed to cause a further increase of plasma corticosterone concentration (Table 3). On the other hand, when ACTH (100 IU/100 g) was given to the diazepam pretreated rats an additional rise of plasma corticosterone (281% compared to control) was obtained, $F(5,37)=180.30$, $p<0.001$. ANOVA indicated that this increase was significantly greater than that obtained after the administration of ACTH alone, $F(5,37)=15.08$, $p<0.05$. ACTH alone increased the corticosterone level, $F(5,37)=91.98$, $p<0.001$, approximately to the same magnitude as the ether stress.

DISCUSSION

A relatively short-lasting stressful procedure (15 min) was sufficient to produce a significant rise of plasma corticosterone concentration, indicating a fast response of the HHA axis to applied ether stress. However, very pronounced activation of the HHA axis was not accompanied by changes in GABA content of the hypothalamus, which differs from the fall in the GABA content obtained by Hahn *et al.* [14]. In our experimental conditions acute ether stress diminished both the AOAA and L-cycloserine induced accumulation of hypothalamic GABA, suggesting that the synthesis of GABA is reduced. If the ether stress induced decrease of GABA accumulation really reflects a decreased GABA synthesis, then the unchanged GABA level after ether stress might be held by corresponding decrease in GABA catabolism. The *in vitro* determined GABA-T activity of the animals exposed to the ether stress failed to confirm such a supposition, because

TABLE 2

EFFECT OF ETHER STRESS ON GAD AND GABA-T ACTIVITY IN THE HOMOGENATES OF RAT HYPOTHALAMI AND ON *IN VITRO* MEASURED SATURATION OF BOTH ENZYMES WITH PYRIDOXAL PHOSPHATE (PLP)

	GAD		
	Activity (nmol GABA/mg protein/5 min)		% of Saturation by PLP
	(−)PLP	(+) PLP	
Control	25.33 ± 0.52	34.63 ± 0.72	71.11 ± 2.17
Ether Stress	28.01 ± 0.77*	37.87 ± 0.75*	73.54 ± 1.68

	GABA-T		
	Activity (nmol product/mg protein/15 min)		% of Saturation by PLP
	(−) PLP	(+) PLP	
Control	84.95 ± 3.47	85.61 ± 2.55	96.43 ± 3.05
Ether Stress	79.70 ± 1.56	85.92 ± 3.77	94.17 ± 3.33

Rats were killed immediately after ether stress (2×2 min within 15 min). The maximal GAD and GABA-T activity was measured by adding saturating concentrations of pyridoxal phosphate (0.1 mM and 0.3 mM respectively) to the reaction mixture. The actual GAD and GABA-T activity of this tissue was measured in the absence of added pyridoxal phosphate. Saturation was calculated for each animal as percentage of actual from the maximal enzyme activity. The results are the mean ± SEM of 10–12 animals in the group.

*$p<0.01$ when compared with the corresponding control group (Student's t-test).

TABLE 3

INFLUENCE OF ETHER STRESS AND ACTH ADMINISTRATION ON PLASMA CORTICOSTERONE OF DIAZEPAM PRETREATED RATS

Treatment	Corticosterone (μg/100 ml plasma)	
Saline	14.02 ± 1.31	(12)
Saline + ether stress	38.99 ± 2.36*	(6)
Saline + ACTH	40.46 ± 1.72*	(6)
Diazepam	40.01 ± 1.98*	(9)
Diazepam + ether stress	37.59 ± 3.86*	(5)
Diazepam + ACTH	53.42 ± 2.24*†	(5)

Diazepam (10 mg/kg) was administered IP 70 min prior to death. Rats were exposed to ether stress (2×2 min within 15 min) or injected IP with ACTH (100 IU/100 g) 55 min after the diazepam or saline administration. The results are the mean ± SEM. Numbers in parentheses represent number of experiments. After the analysis of variance Scheffe's test was applied.

*$p<0.001$ when compared with saline treated control.

†$p<0.01$ when compared with the following groups: saline + ether stress, diazepam, diazepam + ether stress. †$p<0.05$ when compared with saline + ACTH treated group.

ether stress did not have any significant influence on GABA-T activity and on the saturation of GABA-T by its cofactor pyridoxal phosphate. This also rules out the possibility that the ether induced decrease in GABA accumulation is caused by increasing the activity of GABA-T. Our data show that the exposure of animals to the acute ether stress increases the *in vitro* determined activity of hypothalamic GAD. Although this increase was small, it was statistically significant, and it was obtained immediately after the 15 min lasting stress procedure. The increased GAD activity has also been obtained in the ventromedial nucleus of the hypothalamus of rats exposed to long-term immobilization stress (42 days) [10]. A stress induced increase of GABAergic activity has also been suggested by Soubrie *et al.* [32]. The fact that GAD activation was evident in the presence and absence of added pyridoxal phosphate, as well as the fact that *in vitro* determined saturation of GAD with pyridoxal phosphate was equal in the control and ether stressed rats suggests that the stress induced activation of enzyme was achieved by mechanisms other than its increased saturation with pyridoxal phosphate. The finding of the increased GAD activity is in apparent discrepancy with the stress induced diminished accumulation of GABA obtained after blockade of its catabolism. It is possible that the activity of enzymes measured *in vitro* does not always reflect the activity present in the living brain. For example, hormonal influences present *in vivo* may not be detectable *in vitro*. Alternatively, the decreased GABA accumulation seen *in vivo* may reflect phenomena independent of the enzymatic activity, such as decreased availability of precursor or abnormally high rate of release, not balanced by sufficient synthesis.

Recent studies [7,12] have postulated the primary site of action of benzodiazepines on mechanisms involving GABA, supposing them to potentiate GABAergic transmission. Nevertheless, reports of their effects on the HHA activity are conflicting [22,35]. In our experimental conditions acute administration of diazepam (10 mg/kg) to rats produced a significant increase of plasma corticosterone but prevented its further increase by ether stress. The ACTH induced increase of plasma corticosterone in diazepam pretreated animals indicates that the response of the adrenal glands to diazepam was not maximal. Although our finding of a greater corticosterone concentration following the combined treatment with diazepam and ACTH, than following the treatment with ACTH alone (Table 3) might lead to the conclusion that diazepam has enhanced the adrenocortical responsiveness to ACTH, data from the literature [5, 21, 22] suggest the central action of diazepam on the HHA axis. Whether diazepam induced potentiation of GABAergic transmission or some other mechanism is responsible for this action is not clear as yet. Nevertheless, the action of diazepam on other mechanisms controlling the adrenocortical secretion except ACTH cannot be ruled out.

In conclusion, the results might suggest that ether stress activates the hypothalamic GABA system, which is then able to prevent a further response of the HHA axis to stress.

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