Pharmacology Biochemistry & Behavior, Vol. 23, pp. 697–700, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Hypophysial GABA After Ether Stress, Dexamethasone or Inhibition of GABA Catabolism

HARI MANEV AND DANKA PERIČIĆ

Department of Experimental Biology and Medicine, "Rudjer Bošković" Institute
Bijenička cesta 54,41000 Zagreb, P.O.B. 1016, Yugoslavia

Received 28 December 1984

MANEV, H. AND D. PERIČIĆ. *Hypophysial GABA after ether stress, dexamethasone or inhibition of GABA catabolism.* PHARMACOL BIOCHEM BEHAV 23(5) 697–700, 1985.—Ether stress (2×2 min within 15 min) and dexamethasone treatment (1 mg/kg IP; 1, 3 and 12 hours before sacrifice), the procedures supposed to increase the activity of glutamate decarboxylase (GAD) in the hypothalamus, fail to affect the concentration of GABA in the rat hypophysis. Five and/or ten minutes post-mortem an increased GABA level in the hypothalamus and cingulate cortex, and a decreased GABA concentration in the hypophysis was found. Three and four hours after the IP administration of l-cycloserine (50 mg/kg) and l-glutamic acid-γ-hydrazide (160 mg/kg) respectively (both drugs are inhibitors of GABA catabolism) the concentration of GABA raised in all the regions examined. On the basis of studies in the whole gland it might be concluded that the concentration of GABA in the hypophysis depends more on GABA release from extrahypophysial tissue and GABA degradation in the hypophysis than on the extrahypophysial GABA synthesis. Also on the basis of post-mortem studies in the whole gland no indication for the appearance of GABA synthesis in hypophysis could be found.

GABA Hypophysis Hypothalamus Cingulate cortex L-cycloserine L-glutamic acid-γ-hydrazide Dexamethasone Ether stress Post-mortem changes

BESIDES the widespread inhibitory role of γ-aminobutyric acid (GABA) within the central nervous system (CNS) this neurotransmitter also affects the endocrine system (for review see [3]). Although this influence might be achieved by its direct action on the peripheral endocrine structures such as pancreas [24,28], ovary [5], thyroid gland [6], more attention has been paid to the action of GABA at the hypothalamo-hypophysial (HH) level (for review see [21]).

There is presently a strong morphological basis for the regulatory role of GABA at the HH level [25,27]. The hypophysis possesses specific GABA-ergic receptors [4,8] and GABA of the CNS origin is secreted into the hypophysial portal blood [10,15].

Recent investigations have shown that various stressful stimuli increase the activity of hypothalamic glutamate decarboxylase (GAD), the enzyme involved in GABA synthesis [7, 13, 14, 30]. Similar increase of GAD activity has also been obtained in the rat hypothalamus after the onset of suckling [22], or drug-induced secretion of prolactin [16].

Since it has been postulated that the concentration of GABA in the anterior hypophysis might represent a reliable index of the neurotransmitter released from the median eminence [23], it would be logical to expect that the increase in the hypothalamic GAD activity will result in an increased hypophysial GABA content.

To prove this we have chosen two procedures: ether stress and dexamethasone treatment. Ether stress, as previously reported by us [13,14] enhances the hypothalamic GAD activity. The same has been postulated for dexamethasone treatment [1]. Our present investigations have shown that these procedures were unable to change the hypophysial GABA level. Therefore, using various methods of GABA accumulation, we have undertaken further investigations of the GABA system at the HH level, and compared it with the cortical brain GABA system (the cingulate cortex).

METHOD

Male Wistar rats, weighing 160–250 g were caged in groups of three under diurnal lighting conditions. They were given food and water ad lib. At least one hour before the experiment rats were placed into single cages.

L-cycloserine (F. Hoffmann la Roche and Co. A. G., Basel, Switzerland) and l-glutamic acid-γ-hydrazide (GAH; ICN Pharmaceuticals, Cleveland, USA) were administered intraperitoneally (IP) 3 and 4 hours respectively before sacrifice. Dexamethasone (Krka, Novo Mesto, Yugoslavia) was given IP in a dose 1 mg/kg, 1, 3 or 12 hours before death. Control animals were treated IP with saline 1 ml/100 g of body weight.

Ether stress was performed by placing animals 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 stressful procedure the animals were sacrificed. Unstressed rats were undisturbed and killed immediately after removal from the cage.

All animals, except in the experiments where the post-

TABLE 1

THE CONCENTRATION OF GABA IN RAT HYPOPHYSIS FOLLOWING ETHER STRESS OR DEXAMETHASONE ADMINISTRATION

	GABA (nmol/mg protein)	
Control	6.01 ± 0.39	(5)
Ether stress	6.47 ± 0.51	(4)
Control	5.80 ± 0.15	(6)
1 hour after dexamethasone	5.17 ± 0.27	(5)
3 hours after dexamethasone	5.77 ± 0.57	(6)
12 hours after dexamethasone	5.47 ± 0.50	(6)

Rats were sacrificed immediately after the exposure to ether vapour (2×2 min within 15 min), or 1, 3 or 12 hours following the IP injection of dexamethasone (1 mg/kg). Results are the mean ± SEM of (n) experiments. Following ANOVA no significant change was found.

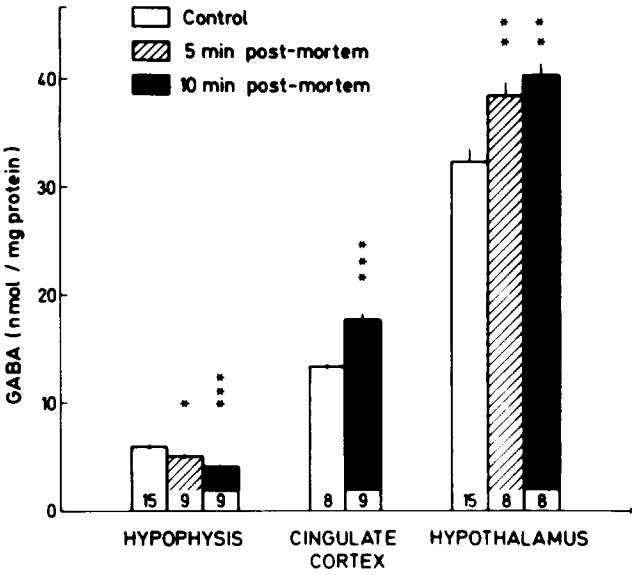

FIG. 1. Post-mortem changes of GABA concentration in the hypophysis, cingulate cortex and hypothalamus. Rats were decapitated and after 5 or 10 min at room temperature the heads were exposed to microwave irradiation. Control animals were killed by microwave irradiation of the brain. The results (data obtained on two consecutive days) are the mean±SEM. Numbers in the bars represent number of experiments. $*p<0.05$, $**p<0.01$, $***p<0.001$ when compared with corresponding control group (Student's t-test).

mortem GABA level was measured, were killed by exposing their heads to a focused beam of microwave irradiation for 5.0 sec [9]. In experiments in which post-mortem change of GABA was measured animals were decapitated, and after 5 or 10 min at room temperature (about 23°C) heads were exposed to microwave irradiation. To avoid the influence of the circadian rhythm on the GABA level all experiments were carried out between 9:00 a.m. and 13:00 p.m.

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 specific brain regions. Punches of hypothalamus and cingulate cortex were obtained as previously described [13,19]. Hypophysis was also taken out and subjected to biochemical analysis. GABA was determined by the previously described [19] enzymatic fluorimetric method. Protein concentrations were determined in 10 μl of the homogenate according to Lowry *et al.* [12]. Statistical analysis of the results was performed by ANOVA and two tailed Student's t-test. The criterion for significance was $p<0.05$.

RESULTS

As shown in Table 1 neither the exposure of rats to ether stress (2×2 min within 15 min), nor the administration of dexamethasone (1 mg/kg; 1,3 or 12 hours before sacrifice) produced any change in the hypophysial GABA content.

When brains of animals decapitated and exposed to microwave after 5 or 10 min at room temperature were compared with brains of animals killed by microwave, decapitated animals had significantly increased GABA levels in the hypothalamus and cingulate cortex (Fig. 1). The average accumulation of GABA in the cingulate cortex was 33.1% 10 min post-mortem, and in the hypothalamus 18.7% and 24.2%, 5 and 10 min post-mortem respectively. On the other side, under the same experimental conditions the concentration of GABA in the hypophysis decreased. This fall was 16.2% and 31.5%, 5 and 10 min post-mortem respectively (Fig. 1).

The calculated disappearance of GABA (mean of the control GABA concentration minus mean of the post-mortem GABA concentration, divided by minutes post-mortem) in the hypophysis was 19.8 nmol GABA/100 mg protein/min, calculated on the basis of 5 min post-mortem decrease, and 18.9 nmol GABA/100 mg protein/min, calculated on the basis of 10 min post-mortem decrease.

The administration of l-cycloserine (50 mg/kg), an inhibitor of GABA:2-oxoglutarate aminotransferase (GABA-T, the enzyme responsible for GABA catabolism) elicited an increase of GABA content in all three regions examined. The concentration of GABA in the hypophysis, cingulate cortex and hypothalamus was 47.5%, 65.2% and 26.3% respectively greater following l-cycloserine administration than following the administration of saline (Fig. 2). GAH, another inhibitor of GABA-T, administered in a dose of 160 mg/kg produced even greater accumulation of GABA in the hypophysis (82.4% when compared with the control group), (Fig. 2).

DISCUSSION

The exposure of rats to the ether vapour is often used as a model of stress. We have recently shown that ether stress increases hypothalamic GAD activity [13,14]. However, this procedure failed to affect the hypophysial GABA level in the present study. We have also previously observed [14] that the activated hypothalamic GAD activity leads to a transient

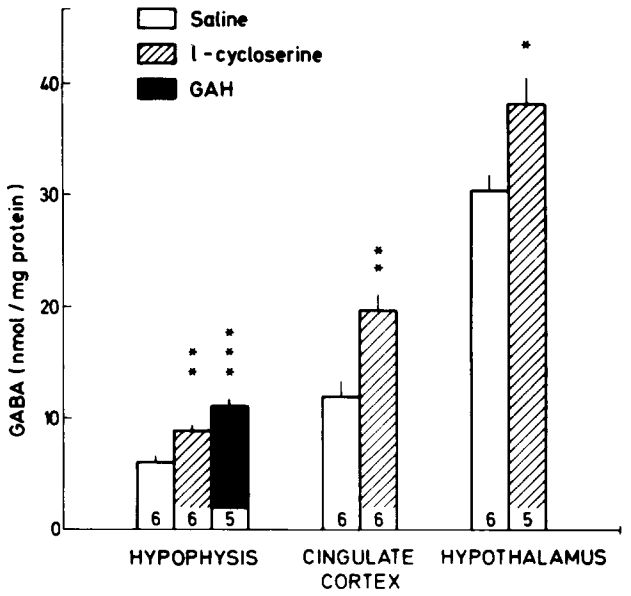

FIG. 2. The effect of l-cycloserine and l-glutamic acid-γ-hydrazide(GAH) on the concentration of GABA in the hypophysis, cingulate cortex and hypothalamus. L-cycloserine (50 mg/kg) and GAH (160 mg/kg) were administered IP 3 and 4 hours respectively prior to death. Results are the mean±SEM. Numbers in the bars represent number of experiments. *$p<0.02$, **$p<0.01$, ***$p<0.001$ when compared with the corresponding saline treated control (Student's t-test).

elevation of hypothalamic GABA concentration 15 min after the termination of stressful procedure. One might presume that in the present study the time period following stress was not long enough (rats were sacrificed at the end of 15 min lasting stressful procedure) to enable the increased hypothalamic GAD activity to elevate hypophysial GABA level. Since Yoneda *et al.* [30] following rather long-lasting stressful procedure (restraint for 3 hours) have found an increased GAD activity and an increased hypothalamic GABA level with no concomitant change in the hypophysial GABA content, this explanation does not seem to be likely. One might conclude therefore that the stress-induced activation of hypothalamic GABA synthesis was not followed by an increased GABA release into the hypophysis.

It has been postulated [1] that dexamethasone may, at least in part, inhibit the secretion of ACTH via the induction of GAD and consecutive rise of GABA level in the CNS. However, we have not noticed any change in the hypophysial GABA concentration following treatment with this drug. Hence, further investigations are necessary to elucidate the postulated influence of dexamethasone on the brain GABA system.

The concentration of GABA in the brain increases rapidly post-mortem [17,18]. The techniques such as rapid freezing of the brain [2,26], focused microwave irradiation [9], or treatment with 3-mercaptopropionic acid a short time before killing [11], have been developed to prevent this post-mortem rise in the brain GABA content. On the other hand, the post-mortem GABA accumulation, like the accumulation of GABA following treatment with drugs capable of inhibiting the catabolism of GABA, has been used for the estimation of GABA synthesis rates in the brain [17, 18, 19, 29]. It is obvious that the post-mortem rise of GABA might occur in the regions where the synthesis of GABA (an active GAD) continues when the catabolism is stopped. This was the case in both brain regions, hypothalamus and cingulate cortex, we have studied. However, during the post-mortem period of 5 and 10 min there was no accumulation of GABA in the hypophysis, moreover a fall in its concentration was found. Although the absence of post-mortem GABA accumulation for some parts of the rat brain cortex has been reported [18], to our knowledge this is the first time that the post-mortem fall in GABA concentration was observed. Hence it might be concluded that the synthesis of GABA in the hypophysis is missing. In accordance with this are the findings of Racagni *et al.* [20] who have reported the absence of GAD activity in the anterior lobe, and only a slight GAD activity in the posterior lobe of the hypophysis. At the same time GABA-T activity, even greater than that found in the hypothalamus, has been found in both lobes of the hypophysis [20]. Vincent *et al.* [27] have reported the presence of GAD in the intermediate lobe of hypophysis. Since we have measured the GABA content of the whole gland it is possible that this GAD activity was unperceptible.

The observed post-mortem disappearance of GABA in the hypophysis also indicates that the post-mortem inhibition of GABA-T was not complete, although degradation of GABA was about 30 times smaller than that observed when measuring the hypophysial [20] or hypothalamic [13] GABA-T activity in vitro. However, even this low GABA-T activity was sufficient to cause a rapid post-mortem degradation of hypophysial GABA, indicating that in order to prevent the post-mortem change of the hypophysial GABA content some of the previously described methods have to be used.

When the degradation of GABA was inhibited by previous l-cycloserine or GAH administration, GABA concentration raised not only in the hypothalamus and cingulate cortex, but also in the hypophysis. The reason for the discrepancy between the hypophysial GABA content following the post-mortem and drug-induced inhibition of GABA catabolism might lie in the fact that the drug-induced inhibition of GABA catabolism occurred in vivo, i.e., with the intact hypothalamo-hypophysial portal circulation.

It has been reported that the electrical stimulation of median eminence [15] as well as the intraventricular administration of ethanolamine-O-sulfate [10] increase the release of GABA into portal blood. Hence, the intact supply of the hypophysis with GABA, along with the blocked GABA degradation, might be responsible for the increased GABA concentration following l-cycloserine or GAH administration. On the other hand, in the post-mortem brain the hypophysial portal circulation was interrupted and the normal supply of hypophysis with GABA discontinued. Thus, although the GABA catabolism was partly suppressed no increase in GABA concentration could occur. Instead, a fall was observed.

In conclusion, the finding based on the studies of post-mortem GABA accumulation indicating the lack of GABA synthesis in the hypophysis, when the whole gland is taken into account, is in corroboration with the literature data concerning the in vitro measurements of the activity of hypophysial enzymes involved in GABA metabolism. Therefore, the origin of the increased hypophysial GABA content following drug-induced inhibition of GABA catabolism should be sought beyond hypophysis, presumably in the hypothalamus. Surprisingly, the hypophysial GABA content was not affected by ether stress or dexamethasone treatment. This might indicate that the increased GAD activity

and the increased GABA synthesis, occurring in conditions such as stress, need not obligatorily be accompanied by the increased GABA release. It also appears thereof that the concentration of hypophysial GABA depends more on GABA release from extrahypophysial tissue and GABA degradation in the hypophysis than on the extrahypophysial GABA synthesis. However, it has to be emphasized that the same conclusions need not obligatorily be valid if particular lobes instead of the whole hypophysis would be studied.

ACKNOWLEDGEMENTS

The authors thank Dr. Petar Polc (F. Hoffmann la Roche and Co. A. G., Basel, Switzerland) for the supply of l-cycloserine, and Mrs. Ivanka Fresl for her skilled technical assistance.

REFERENCES

1. Ács, Z. and E. Stark. Possible role of gamma-aminobutyric acid synthesis in the mechanism of dexamethasone feedback action. *J Endocrinol* **77:** 137–141, 1978.
2. Alderman, J. L. and M. K. Schellenberg. γ-Aminobutyric acid (GABA) in the rat brain: Re-evaluation of sampling procedures and the postmortem increase. *J Neurochem* **22:** 937–940, 1974.
3. DeFeudis, F. V. GABA and endocrine regulation—Relation to neurologic-psychiatric disorders. *Neurochem Int* **6:** 1–16, 1984.
4. Demeneix, B. A., E. Desaulles, P. Feltz and J. P. Loeffler. Dual population of $GABA_A$ AND $GABA_B$ receptors in rat pars intermedia demonstrated by release of αMSH caused by barium ions. *Br J Pharmacol* **82:** 183–190, 1984.
5. Erdö, S. L. and A. László. High specific γ-aminobutyric acid binding to membranes of the human ovary. *J Neurochem* **42:** 1464–1467, 1984.
6. Gebauer, H. and M. A. Pabst. Autoradiographic localization of ^{3}H-GABA uptake in the thyroid gland of the rat. *Cell Tissue Res* **220:** 873–879, 1981.
7. Gottesfeld, Z., R. Kvetňanský, I. J. Kopin and D. M. Jacobowitz. Effects of repeated immobilization stress on glutamate decarboxylase and choline acetyltransferase in discrete brain regions. *Brain Res* **152:** 374–378, 1978.
8. Grandison, L., F. Cavagnini, R. Schmid, C. Invitti and A. Guidotti. γ-Aminobutyric acid- and benzodiazepine-binding sites in human anterior pituitary tissue. *J Clin Endocrinol Metab* **54:** 597–601, 1982.
9. Guidotti, A., D. L. Cheney, M. Trabucchi, M. Doteuchi and C. Wang. Focussed microwave radiation. A technique to minimize post mortem changes of cyclic nucleotides, dopa and choline and to preserve brain morphology. *Neuropharmacology* **13:** 1–7, 1974.
10. Gudelsky, G. A., J. A. Apud, C. Masotto, V. Locatelli, D. Cocchi, G. Racagni and E. E. Müller. Ethanolamine-O-sulfate enhances γ-aminobutyric acid secretion into hypophysial portal blood and lowers serum prolactin concentrations. *Neuroendocrinology* **37:** 397–399, 1983.
11. Lindgren, S. Effects of the glutamic acid decarboxylase inhibitor 3-mercaptopropionic acid on the synthesis of brain GABA in vivo and postmortally. *J Neural Transm* **58:** 75–82, 1983.
12. Lowry, O. H., N. J. Rosebrough. A. L. Farr and R. J. Randall. Protein measurement with the folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
13. Manev, H. and D. Peričić. Hypothalamic GABA system and plasma corticosterone in ether stressed rats. *Pharmacol Biochem Behav* **18:** 847–850, 1983.
14. Manev, H. and D. Peričić. Changes in the GABA-ergic system of ether stressed rats. *Iugoslav Physiol Pharmacol Acta* **19:** 110–113, 1983.
15. Mitchell, R., G. Grieve, R. Dow and G. Fink. Endogenous GABA receptor ligands in hypophysial portal blood. *Neuroendocrinology* **37:** 169–176, 1983.
16. Nicoletti, F., G. Clementi, A. Prato, P. L. Canonico, L. Rampello, F. Patti, R. M. Di Giorgio and U. Scapagnini. Effects of hyper- and hypoprolactinemia on glutamate decarboxylase activity in medial basal hypothalamus of male rat. *Neuroendocrinology* **36:** 13–16, 1983.
17. Patel, A. J., A. L. Johnson and R. Balázs. Metabolic compartmentation of glutamate associated with the formation of γ-aminobutyrate. *J Neurochem* **23:** 1271–1279, 1974.
18. Peričić, D., N. Eng and J. R. Walters. Post-mortem and aminooxyacetic acid-induced accumulation of GABA: Effect of gamma-butyrolactone and picrotoxin. *J Neurochem* **30:** 767–773, 1978.
19. Peričić, D., J. R. Walters and T. N. Chase. Effect of diazepam and pentobarbital on aminooxyacetic acid-induced accumulation of GABA. *J Neurochem* **29:** 839–846, 1977.
20. Racgani, G., J. A. Apud, C. Civati, D. Cocchi, F. Casanueva, V. Locatelli, G. Nicticò and E. E. Müller. Neurochemical aspects of GABA and glutamate in the hypothalamo-pituitary system. In: *GABA and Benzodiazepine Receptors, Advances in Biochemical Psychopharmacology*, vol 26, edited by E. Costa, G. Di Chiara and G. L. Gessa. New York: Raven Press, 1981.
21. Racagni, G., J. A. Apud, D. Cocchi, V. Locatelli and E. E. Müller. GABAergic control of anterior pituitary hormone secretion. *Life Sci* **31:** 823–838, 1982.
22. Racagni, G., J. A. Apud, E. Iuliano, D. Cocchi, V. Locatelli and E. E. Müller. Anterior pituitary GABA receptors in relation to prolactin secretion. In: *CNS Receptors: From Molecular Pharmacology to Behavior, Advances in Biochemical Psychopharmacology*, vol 37, edited by P. Mandel and F. V. DeFeudis. New York: Raven Press, 1983.
23. Racagni, G., J. A. Apud, V. Locatelli, D. Cocchi, G. Nisticò, R. M. Di Giorgio and E. E. Müller. GABA of CNS origin in the rat anterior pituitary inhibits prolactin secretion. *Nature* **281:** 575–578, 1979.
24. Reusens-Billen, B., X. Pirlot, C. Remacle, J. J. Hoet and M. De Gasparo. Localization of GABA high-affinity binding sites in the pancreas of neonatal rat. *Cell Tissue Res* **235:** 503–508, 1984.
25. Tappaz, M. L., M. Wassef, W. H. Oertel, L. Paut and J. F. Pujol. Light- and electron-microscopic immunocytochemistry of glutamic acid decarboxylase (GAD) in the basal hypothalamus: Morphological evidence for neuroendocrine γ-aminobutyrate (GABA), *Neuroscience* **9:** 271–287, 1983.
26. Veech, R. L., R. L. Harris, D. Ve Loso and E. H. Veech. Freeze-blowing: A new technique for the study of brain *in vivo*. *J Neurochem* **20:** 183–188, 1973.
27. Vincent, S. R., T. Hökfelt and J. Y. Wu. GABA neuron systems in hypothalamus and the pituitary gland. Immunohistochemical demonstration using antibodies against glutamate decarboxylase. *Neuroendocrinology* **34:** 117–125, 1982.
28. Vincent, S. R., T. Hökfelt, J. Y. Wu, R. P. Elde, L. M. Morgan and J. R. Kimmel. Immunohistochemical studies of the GABA system in pancreas. *Neuroendocrinology* **36:** 197–204, 1983.
29. Walters, J. R., N. Eng, D. Peričić and L. P. Miller. Effects of aminooxyacetic acid and l-glutamic acid-γ-hydrazide on GABA metabolism in specific brain regions. *J Neurochem* **30:** 759–766, 1978.
30. Yoneda, Y., K. Kanmori, S. Ida and K. Kuriyama. Stress-induced alterations in metabolism of γ-aminobutyric acid in rat brain. *J Neurochem* **40:** 350–356, 1983.