12. M. Novac, J. Lipid Res., 6, 431 (1965).
13. S. G. Shimada and J. T. Stitt, Can. J. Physiol. Pharmacol., 61, 977 (1983).
14. R. E. Smith and D. J. Hoijer, Physiol. Rev., 42, 60 (1962).

MONOAMINES AND PEPTIDERGIC GOMORI-POSITIVE NEUROSECRETORY CELLS OF THE PARAVENTRICULAR NUCLEUS OF THE RAT HYPOTHALAMUS DURING CHRONIC ALCOHOL CONSUMPTION

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The effect of chronic alcohol intake on metabolism and concentrations of monoamines in the hypothalamus have been studied in some investigations [1, 2, 7, 9, 12], and secretory activity of Gomori-positive neurosecretory cells (NSC) of the hypothalamus [5], producing nonapeptide neurohormones and possessing a rich monoaminergic innervation [4] in others.

The aim of this investigation was to make a parallel study, in one experiment, of the concentrations of individual monoamines, the fluorescence of monoaminergic structures, and the functional state of peptidergic Gomori-positive NSC of the paraventricular nucleus (PVN) in the hypothalamus of rats during chronic (6 months) alcoholization. Data on the functional state of the monoaminergic and peptidergic systems of the hypothalamus, the center for neuroendocrine regulation, are of great importance for the analysis of neuroendocrine disturbance during chronic alcohol exposure, more especially since the role of disturbances of the brain monoaminergic systems in the pathogenesis of chronic alcoholism has been discovered [1, 2].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats fed on a standard diet but allowed to drink only a 20% solution of ethanol for 6 months. For the next two weeks the rats had the choice of drinking ethanol or water. Experimental rats were divided into two groups: 1) preferring alcohol, 2) preferring water. Rats given only water to drink for 6.5 months served as the control. All rats weighing 350-400 g were decapitated in the fall. Concentrations of dopamine (DA), noradrenalin (NA), and serotonin (5-HT) in the hypothalamus were determined by a modified method [14]. The significance of the results was determined by Student's test. Monoaminergic structures of the hypothalamus were revealed by the fluorescence-histochemical method of Falck and Hillarp (in 5 rats in each experimental group and in the control). The intensity of fluorescence of the monoamines was estimated by counting the number of varicose thickenings of terminals of monoaminergic fibers in the hypothalamic nuclei. Some of the sections treated by the Falck–Hillarp method were stained with paraldehyde–fuchsin by the Gomori–Gabe method and counterstained with Heidenhain's azan. The quantity of Gomori-positive neurosecretory material in the bodies and axons of the NSC in PVN was then determined in the preparation. The estimation was done visually, using a five-point system, with an accuracy of 0.5 point.

EXPERIMENTAL RESULTS

The intensity of green fluorescence of noradrenalin- and dopaminergic nerve fibers in the hypothalamic nuclei of the rats of group 1 was statistically significantly lower than in the

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TABLE 1. Levels of Hypothalamic Monoamine (in μg/g tissue) after Chronic Alcohol Consumption by Rats with Different Addiction to Alcohol (in % concentration of monoamine in hypothalamus of control rats, taken as 100%)

Group of rats	NA	DA	5-HT
Control	0,681±0,018	0,783±0,034	0,814±0,048
$n = 54$	%	%	%
1. $n = 30$	88,1*	36,3**	149,8**
2. $n = 23$	124,2**	50,4**	117,6

Legend. n) Number of animals. *P < 0.05, **P < 0.01.

control, especially in the region of PVN (Fig. 1, a and b). This fact correlates with lowering of the NA and DA levels in the hypothalamus of this group of rats (Table 1). A fall in the NA level in the hypothalamus of the rats of group 1 was accompanied by a marked increase in the 5-HT concentration (Table 1). In the dorsomedial, ventromedial, and arcuate nuclei of the rats of group 1 it was possible to see cells containing granules in their cytoplasm with yellow fluorescence, characteristic of 5-HT; these cells, evidently accumulating 5-HT, were not found in these nuclei in the rats of group 2 or in the control. On the other hand, in the rats of group 2 the intensity of green fluorescence of large numbers of nerve fibers in the hypothalamus, which are particularly marked in PVN and in fibers of the median forebrain bundle, was considerably higher than that in the rats of group 1 and in the control. In the hypothalamus of the rats of group 2, on the other hand, the NA level was significantly raised but the DA level was lowered (Table 1). Large palely stained NSC predominated in PVN of the rats of group 1. The content of neurosecretory material in the perikarya of NSC was very small. The numerous axons of these cells were widened and packed with neurosecretory material (Fig. 2b). By contrast with the control (Fig. 2a), darkly stained NSC predominated in PVN of the rats of group 2. Much neurosecretory material was observed in both the perikarya and the axons of NSC in PVN. A parallel was observed between changes in the content of Gomori-positive and immunoreactive material, revealing nonapeptide neurohormones and those of them which carried neurophysin. Thus processes of synthesis and transport of nonapeptide neurohormones in NSC can be judged from the content of Gomori-positive material in the bodies and processes of NSC [3].

When the effect of chronic alcohol consumption on the monoaminergic systems of the hypothalamus is observed [13], the sensitivity of its dopaminergic systems stands out particularly clearly [7], in agreement with changes in the DA level in the present experiments (TABLE 1). Acetaldehyde, an active metabolite of alcohol, affects the metabolism of DA [9] and its synthesis in the brain, disturbing the sensitivity of DA receptors [7, 10]. It is not yet known whether alcohol has any direct action on the dopaminergic systems, or whether its action is mediated through NA and the 5-HT system [7, 11]. The DA-neurons of the hypothalamus have been shown to receive a 5-HT innervation and to have 5-HT receptors. Under these circumstances the 5-HT innervation has an inhibitory action on activity of the DA system of the hypothalamus [11], and this may also have taken place in the hypothalamus of the rats of group 1 (Table 1). The fall in the DA concentration in the hypothalamus of the rats of group 2 was probably due to transformation of DA into NA, as is indicated both by the increased NA concentration in them (Table 1) and by the increased intensity of fluorescence of NA fibers in PVN, and along the course of fibers of the median forebrain bundle — the course of the NA fibers of the hypothalamus. Alcohol also affects the NA systems of the brain and, in particular, the NA systems of the hypothalamus (Fig. 1b, Table 1) and neurons of the locus coeruleus [8], the main source of the NA innervation of hypothalamic structures, and especially of PVN. In this connection the change in functional activity of the peptidergic Gomori-positive NSC in PVN in chronically alcoholized animals is not surprising. Disturbance of the balance between synthesis of nonapeptide neurohormones in the perikarya and their transport along the axons of NSC within PVN was more marked in the rats which preferred alcohol (activation of synthesis and inhibition of transport of neurohormones; Fig. 2b), and was evidently associated with weakening of the inhibitory effect of the NA innervation and nonapeptide synthesis in he perikarya of NSC [4].

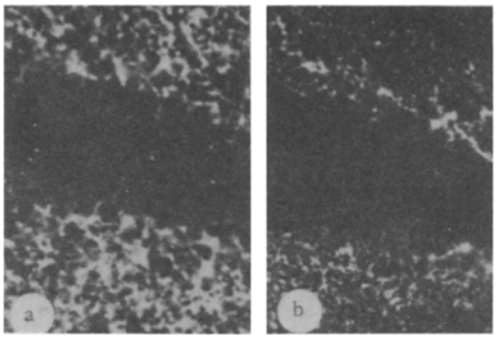

Fig. 1

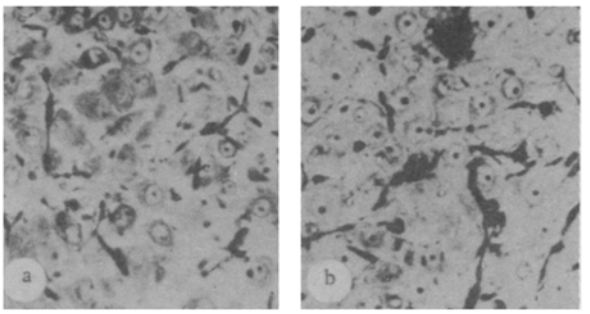

Fig. 2

Fig. 1. Fluorescence of noradrenergic fibers in PVN region after chronic alcohol intake in rats with high preference for alcohol. Reduction of intensity of fluorescence of NA terminals around nonfluorescent cells in PVN can be seen in experimwnt (b) compared with bright fluorescnece of NA terminals in control (a). Falck–Hillarp fluorescence method. Ocular, homal 3, objective 40.

Fig. 2. Cells of PVN after chronic alcohol intake by rats with high preference for alcohol. Numerous expansions of processes of PVN neurons and increased accumulation of Gomori-positive neurosecretory material in them can be seen in experiment (b) compared with control. Paraldehyde-fuchsin, ocular 3.2, objective 25.

However, not only the peptidergic systems of the hypothalamus are under the control of monoaminergic systems. The peptidergic systems of the hypothalamus also influence functional activity of the monoaminergic systems [15]. Chronic alcoholization thus leads to a disturbance of the function of the monoaminergic and peptidergic systems of the hypothalamus. In rats preferring alcohol, unlike those preferring water, the 5-HT level was raised and the NA level lowered, and these changes correlated with the decrease in fluorescence of the NA structures in the hypothalamus, accompanied by a marked disturbance of transport of nonapeptide neurohormones along axons of NSC in the region of PVN.

LITERATURE CITED

1. I. P. Anokhina, and B. M. Kogan, Zh. Nevropatol. Psikhiat., 75, 1874 (1975).
2. E. A. Gromova, N. V. Bobkova, L. A. Plakkhinas, et al., Neirokhimiya, 2, 119 (1983).
3. P. P. Denisenko, M. S. Konstantinova, and T. G. Naimova, Farmakol. Toksikol., 16, 618 (1978).
4. M. S. Konstantinova, Byull. Éksp. Biol. Med., 88, 518 (1979).
5. A. N. Yavorskii, Probl. Endokrinol., No. 5, 63 (1978).
6. R. C. Aloia, J. Paxton, J. S. Daviau, et al., Life Sci., 36, 1003 (1985).
7. M. L. Barbaccia, A. Bosio, L. Lucchi, et al., Life Sci., 30, 2163 (1982).
8. J. Brick and L. A. Pohorecky, Life Sci., 35, 207 (1984).
9. R. A. Dietrich and V. G. Erwin, Science, 67, 1005 (1970).
10. P. L. Hoffman and V. Tabakoff, Nature, 286, 551 (1977).
11. C. O. Lynch, M. D. Johnson, and W. R. Crowley, Life Sci., 35, 1481 (1984).
12. M. A. Mena and E. Herrera, J. Neurol. Transmiss., 47, 227 (1980).
13. H. Öhlin, R. Hörlin, J. Wadstein, et al., Drug Alcohol Depend., 5, 181 (1980).
14. M. Schlumpf, W. Lichtensteiger, H. Langemann, et al., Biochem. Pharmacol., 23, 2437 (1974).
15. G. Telegdy and G. L. Kovacs, Central Nervous System Effects of Hypothalamic Hormones and Other Peptides, New York (1979), pp. 189-205.