2. F. Z. Meerson, Pathogenesis and Prevention of Stress-Induced and Ischemic Heart Damage [in Russian], Moscow (1984).
3. M. G. Panchenko, G. N. Baldenkov, and V. A. Tkachuk, Byull. Vses. Kardiol. Nauch. Tsent., No. 1, 31 (1984).
4. M. Billah, E. Lapetina, and P. Cuatrecasas, J. Biol. Chem., 256, 5399 (1981).
5. S. G. Dahla, E. Hough, and P.-A. Hals, Biochem. Pharmacol., 35, 1263 (1986).
6. W. R. Dayton and J. V. Schollmeyer, J. Mol. Cell. Cardiol., 12, 533 (1980).
7. A. Fleckenstein, Symposium on Assessment of Isoptain Treatment Effects. A. L. Myasnikov Institute of Cardiology, Academy of Medical Sciences of the USSR, Moscow (1969), pp. 12-19.
8. R. F. Gilmor, P. Douglas, and P. Zipes, Am. J. Cardiol., 55, 89B (1985).
9. M. M. Ho, D. J. Scales, and G. Inesi, Biochim. Biophys. Acta, 730, 64 (1983).
10. P. D. Lew and T. P. Stossel, J. Clin. Invest., 67, 1 (1981).
11. R. C. Ruth, K. Owins, and W. B. Weglicki, J. Pharm. Exp. Ther., 272, 361 (1980).
12. T. Toyo-Oka and T. H. Masaki, J. Mol. Cell. Cardiol., 8, 769 (1979).

EFFECT OF SODIUM HYDROXYBUTYRATE ON CATECHOLAMINE AND SEROTONIN LEVELS AND MONOAMINE OXIDASE ACTIVITY IN ALCOHOLICS

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Modulation of activity of the GABA-benzodiazepine receptor complex is not the only mechanism involved in realization of the anxiolytic effect. Drugs affecting the noradrenergic, dopaminergic, and serotoninergic systems of the brain also have an anxiolytic action [5, 10, 12]. Sodium hydroxybutyrate, the sodium salt of γ-hydroxybutyric acid (GHBA), is a GABA metabolite in brain tissue and gives GABA-positive effects [8]. Meanwhile GHBA causes accumulation of noradrenalin, blocks the conduction of impulses along dopaminergic nerve fibers, accelerates serotonin metabolism, and inhibits monoamine oxidase (MAO) activity in the CNS [7, 13-15]. The close similarity of the chemical structures of sodium hydroxybutyrate and GHBA suggests that the mechanism of action of this compound is based on the effects of GHBA. Sodium hydroxybutyrate has been used in the treatment of drug addiction as a substance to abolish addiction to alcohol and to treat the alcohol withdrawal syndrome [1]. The aim of this investigation was to study the psychotropic effect of sodium hydroxybutyrate and to compare it with its effect on plasma adrenalin (A), dopamine (DA), noradrenalin (NA), and serotonin (5-HT) levels and on platelet MAO activity in alcoholics.

EXPERIMENTAL METHOD

The subjects were 36 patients with chronic alcoholism in stage II (average age 35.1 ± 6.1 years) 2 weeks after abolition of the alcohol withdrawal syndrome. During the 10 days before the investigation the patients received no medication. A mean single dose of 2 g (40 ml of a 5% sugar syrup solution) was used as the test dose of sodium hydroxybutyrate. The psychotropic effect of the drug was evaluated by a clinical-descriptive method and recorded on a 4-point psychometric scale: 0) no effect, 1) weak effect, 2) moderate effect, 3) strong effect. Plasma levels of A, NA, DA, and 5-HT and platelet MAO type B activity (MAO-B) were

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TABLE 1. Effect of Sodium Hydroxybutyrate on Plasma Catecholamine Levels in Alcoholics

Group of patients	Number of patients	A, pg/ml		DA, pg/ml		NA, pg/ml	
		background	3 h	background	3 h	background	3 h
As a whole	37	35,4±3,6	25,9±1,1*	161,3±46,3	87,9±27,9	143,7±22,3	134,6±28,1
Sensitive	27	36,9±4,8	26,2±1,5*	200,7±58,9	79,3±30,2*	122,2±21,6	146,2±32,7
Insensitive	9	28,9±3,9	25,0±0,0	31,7±12,8**	101,8±157,2	208,2±62,9	76,8±34,1
Placebo	20	45,1±11,6	32,2±6,1	84,6±23,3	82,4±23,9	226,3±28,6	244,0±58,9

Legend. Here and in Table 3: *p < 0.05 compared with background value; **p < 0.05 compared with background value in sensitive patients.

TABLE 2. Effect of Sodium Hydroxybutyrate on Plasma Serotonin Level in Alcoholics

Group of patients	Number of patients	5-HT, ng/ml	
		background	3 h
As a whole	32	73,6±5,9	76,3±8,2
Sensitive	23	79,2±7,5	80,2±9,3
Insensitive	9	59,3±7,7	62,0±16,8
Placebo	16	81,5±10,5	101,6±16,4

determined before and 3 h after administration of the compound. The patients were tested at 9.30 a.m., 1.5 h after a standard breakfast. Blood (7 ml) was taken from the cubital vein. The free plasma catecholamine level was determined by high-performance liquid chromatography with fluorometric detection [9]. Catecholamines were isolated from plasma by precipitation on alumina followed by elution with an acid eluent [11]. The supernatant of the plasma, after separation of the catecholamines, was acidified to pH 3.0 and, after separation of the residue, was used for fluorometric determination of serotonin ($\lambda_{extinction}$ = 320 nm, $\lambda_{emission}$ = 350 nm). The method of determination of MAO-B activity was as follows: 1) packed platelets were isolated by a modified method [4]; whole heparinized blood was layered above a solution of Ficoll (ρ = 1.074 g/ml) and centrifuged for 10 min at 1500g; the supernatant, containing only platelet suspension, was transferred to a test tube and centrifuged for 30 min at 6000g. Only platelets were thrown down in the residue; 2) MAO-B activity was determined [6] by a modified method [3]: the detergent Triton X-100 was added to a final concentration of 25% for solubilization of the platelets; 3) the protein concentration was determined by the Lowry–Cioccalteu method [2], using serum albumin to plot the calibration curve.

EXPERIMENTAL RESULTS

The concentration of A and MAO-B activity in the blood plasma of the alcoholics were reduced 3 h after administration of sodium hydroxybutyrate, whereas concentrations DA, NA, and 5-HT were unchanged (Tables 1, 2, and 3).

The psychotropic effect after administration of sodium hydroxybutyrate was observed in 27 patients (76%). After administration of the preparation to the patients their excitability, internal stress, and manifestations of anxiety were reduced and their background mood was stabilized. The action of the compound began after 30-40 min and the effect lasted about 4 h. Side effects of the drug were observed in seven patients (19%): elevation of the mood was observed for 15 min, 10-15 min after taking the compound, and was accompanied by mild dizziness, "noises in the head," and nausea and vomiting in some cases. The intensity of the psychotropic effect on the psychometric scale was 1.7 ± 1.2 points. In nine patients (24%) no clinical manifestations of the psychotropic effect were found. Depending on the presence or absence of a psychotropic effect after a test dose of sodium hydroxybutyrate, the group of patients was divided into two subgroups: sensitive and insensitive to the drug.

Under the influence of sodium hydroxybutyrate plasma concentrations of A and DA fell in patients sensitive to it, whereas concentrations of NA and 5-HT were unchanged (Tables 1 and 2). In patients resistant to sodium hydroxybutyrate, concentrations of monoamines in the blood plasma were unchanged (Tables 1 and 2).

In emotional stress the A level in the blood and CNS is known to be raised [12]. The lowering of the A level which we found under the influence of sodium hydroxybutyrate may perhaps be one mechanism of its stress-protective action.

TABLE 3. Effect of Sodium Hydroxybutyrate on Platelet MAO Activity in Alcoholics

Group of patients	Number of patients	MAO activity, μmoles/mg protein (0.75 g)	
		background	3 h
As a whole	12	0,15±0,04	0,08±0,01*
Placebo	7	0,08±0,02	0,08±0,01

We found that the psychotropic effect of sodium hydroxybutyrate is determined to some degree by the background DA level ($r = +0.5$, $p < 0.001$). In patients sensitive to sodium hydroxybutyrate the background plasma DA levels were higher than in resistant patients (Table 1). Lowering of the plasma DA level in patients sensitive to sodium hydroxybutyrate is in agreement with experimental data on depression of dopamine impulsation by the action of GHBA [13]. The decrease in MAO-B activity which we found near the influence of sodium hydroxybutyrate is in agreement with experimental results showing reduced brain MAO activity under the influence of GHBA [14].

The results of this investigation indicate that the dopaminergic system may be involved in the realization of the psychotropic effect of sodium hydroxybutyrate. The role of lowering of activity of the dopaminergic system in the development of the tranquilizing effect is confirmed by the effectiveness of small doses of haloperidol, a dopamine receptor antagonist, in abolishing anxiety disorders [10].

Thus the leading role in the realization of the psychotropic effect of sodium hydroxybutyrate is played by the GABA-ergic and dopaminergic systems, whereas the noradrenergic and serotoninergic systems had no evident role to play. Recording changes in the plasma DA level with time under the influence of sodium hydroxybutyrate can be used as an individual test for predicting the clinical effect of this substance in the treatment of alcoholics.

LITERATURE CITED

1. V. A. Aleksandrov and G. A. Livanov, Problems in Alcoholism [in Russian], Leningrad (1971), pp. 1-16.
2. D. Bailey, Methods in Protein Chemistry [Russian translation], Moscow (1965).
3. H. Friemel (ed.), Immunologischer Arbeitsmethoden, Fischer, Jena (1976).
4. V. N. Orekhovich (ed.), Modern Methods in Chemistry [in Russian], Moscow (1977).
5. D. S. Charney, Psychopharmacol. Bull., 22, 507 (1986).
6. A. Georgotas, R. E. McCue, and T. Cooper, Am. J. Psychiat., 144, 338 (1987).
7. G. L. Gessa, L. Vargin, F. Crabai, et al., Life Sci., 5, 1921 (1966).
8. L. Hosli, E. Hosli, R. Lennean, et al., Neurosci. Lett., 37, 257 (1983).
9. T. Ishimitsu and S. Hirose, J. Chromatogr., 337, 239 (1985).
10. H.-S. Moller, Med. Clin., 81, 385 (1986).
11. K. Mori, J. Chromatogr., 218, 631 (1981).
12. D. E. Redmond, Phenomenology and Treatment of Anxiety, New York (1979), p. 153.
13. O. C. Snead, Life Sci., 20, 1935 (1977).
14. P. F. Spano, A. Tagliamonte, P. Tagliamonte, and G. L. Gessa, J. Neurochem., 18, 1831 (1971).
15. P. F. Spano and P. Przegalsniski, Pharmacol. Res. Commun., 5, 55 (1973).