LITERATURE CITED

1. I. S. Morozov, Farmakol. Toksikol., No. 5, 540 (1980).
2. I. S. Morozov and D. D. Pukhov, Byull. Éksp. Biol. Med., No. 10, 500 (1979).
3. I. S. Morozov and G. S. Pukhova, in: Pharmacological Regulation of Fatigue Processes [in Russian], Moscow (1982), p. 102.
4. G. S. Pukhova, I. S. Morozov, and E. R. Ivanov, Uch. Zap. Tartu. Univ., No. 606, 148 (1982).
5. J. G. Bainbridge and D. T. Greenwood, Neuropharmacology, 10, 453 (1971).
6. W. C. Bowman and M. W. Nott, Pharmacol. Rev., 21, 27 (1969).
7. C. Dahlöf, G. Engberg, and T. H. Svensson, Arch. Pharmakol. Exp. Pathol., 317, 26 (1981).
8. E. Fifkova and J. Marsala, in: Electrophysiological Methods in Biological Research, Prague (1960), p. 426.
9. R. N. Hughes, Life Sci., 29, 1089 (1981).
10. H. Kuribara, Pharmacol. Biochem. Behav., 8, 537 (1978).
11. R. McCarty and L. J. Kopin, Hormones Behav., 11, 248 (1978).
12. M. Sidman, Science, 118, 157 (1953).

EFFECT OF TSH RELEASING HORMONE AND ITS ANALOGS WITH DIFFERENT HORMONAL ACTIVITY ON SOME PHARMACOLOGICAL EFFECTS OF ETHANOL

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Thyroid stimulating hormone releasing hormone (TSHRH), the hypothalamic releasing hormone which induces liberation of thyroid stimulating hormone (TSH) and prolactin, has a broad spectrum of biological activity. It not only has purely hormonal properties, but also exhibits antagonism against the hypnotic and hypothermic effects of ethanol and barbiturates, and also against the cataleptic action of β-endorphin [3, 6]; a possible extrahypophyseal mechanism of realization of these effects, moreover, has been suggested [4].

To study relations between the hormonal and nonhormonal components in the spectrum of pharmacological action of TSHRH, a comparative study was made of the "antialcoholic" properties of TSHRH and its analogs with modified hormonal activity.

EXPERIMENTAL METHOD

The following peptides were studied in the experiments: TSHRH, L-pyroglutamyl-L-seryl-L-leucinamide (TSHRH-2), an analog with no effect on TSH secretion, but which reduces prolactin secretion, and the methyl ester of TSHRH (TSHRH-3), with sharply reduced releasing activity with respect to both hypophyseal hormones [2]. All compounds were synthesized in the laboratory of Protein Hormone Chemistry, Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR. Experiments were carried out on noninbred male albino mice weighing 20-25 g and kept on a pellet diet.

The narcotic action of ethanol was determined by measuring the length of time the mice spent in the side position after intraperitoneal injection in a dose of 4.75 g/kg in the form of a 25% solution. The rectal temperature (t°C) was measured by means of a thermometer (from Nihon Kohden, Japan) 10 min before and 30 min after intraperitoneal injection of 3.5 g/kg of

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25% ethanol ($\Delta t°C$ was defined as the difference between the first and second values). The number of mice which fell off the revolving rod (speed 11 rpm) was recorded during 1 min, 15 min after intraperitoneal injection of 3.5 g/kg of 25% ethanol. TSHRH and its analogs were dissolved immediately before use in physiological saline at the rate of 0.1 ml solution per 10 g body weight, and injected simultaneously with ethanol in a dose of 20 mg/kg (about IC_{50} of TSHRH), for analysis of the duration of ethanol narcosis, and in a dose of 5 mg/kg (about IC_{50} of TSHRH) when temperature was measured. The maximal dose of TSHRH and its analogs for use in the revolving rod test did not exceed 5 mg/kg (intraperitoneally), for if the dose was increased the animals began to jump actively off the rod. The development of tolerance was recorded both by the revolving rod test and by the hypothermic effect during twice daily injection of 25% ethanol in a dose of 3.5 g/kg simultaneously with TSHRH or its analogs in a dose of 5 mg/kg.

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The effect of TSHRH and its analogs on the acute effects of ethanol is illustrated graphically in Fig. 1. TSHRH and its derivatives with modified hormonal activity, in a dose of 5 mg/kg, had no effect on the ability of the animals to remain on the revolving rod after injection of 3.5 g/kg ethanol (Fig. 1C; $P > 0.05$). However, in the same dose, TSHRH and TSHRH-2 significantly prevented the fall of rectal temperature caused by injection of 3.5 g/kg ethanol (Fig. 1B). The fall of rectal temperature after simultaneous injection of TSHRH and of 4.75 g/kg ethanol was 28.8 ± 4.91% of the control ($P < 0.01$), whereas after injection of TSHRH-2 it was 63.46 ± 7.25% ($P < 0.05$). TSHRH-3 had virtually no effect on the parameter tested (95.4 ± 9.7%, $P > 0.05$). Shortening of the duration of ethanol narcosis (Fig. 1A) was observed after injection of all three substances, but the effect of TSHRH-3 (59.24 ± 18.53%) compared with the control was not statistically significant ($P > 0.05$). The ability of TSHRH and TSHRH-2 to modify the duration of ethanol narcosis was about equal: 35.23 ± 10.42% ($P < 0.05$) and 36.33 ± 7.47% ($P < 0.05$), respectively, compared with the control group.

None of the preparations affected the rate of development of tolerance to ethanol, as revealed by the revolving rod test (Fig. 2) and the fall of rectal temperature (Fig. 3). By both tests tolerance appeared on the 3rd day of injection in both the control and experimental groups. Tolerance was considered to have arisen if no significant difference was present in the values of the parameters compared with the previous day of injection. Nevertheless it must be pointed out that when tolerance to the hypothermic effect of ethanol developed, especially in the case of combined administration with TSHRH, differences in the absolute values of $\Delta t°C$ compared with the control group remained statistically significant on all days of the experiment. A similar rule also was observed during the study of the action of TSHRH on the development of tolerance to ethanol as shown by the revolving rod test.

When these data are analyzed it must be recalled that the analeptic properties of TSHRH and its analogs are virtually independent of their hormonal activity. It will be evident that this property of TSHRH is not connected with its inducing action either on TSH or on prolactin, for TSHRH-2, which does not affect TSH secretion and reduces prolactin secretion, has an analeptic action relative to ethanol narcosis that is equal in magnitude and direction. These results suggest involvement of extrahypophyseal mechanisms in the realization of the analeptic

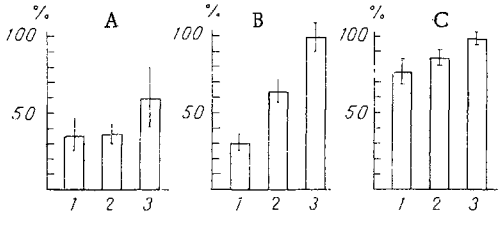

Fig. 1. Action of TSHRH and its analogs on acute effects of ethanol (in % of control, taken as 100). A) Duration of ethanol narcosis; B) $\Delta t°C$; C) number of falls from revolving rod. 1) TSHRH, 2) TSHRH-2, 3) TSHRH-3.

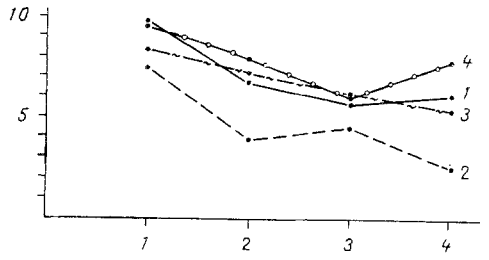

Fig. 2

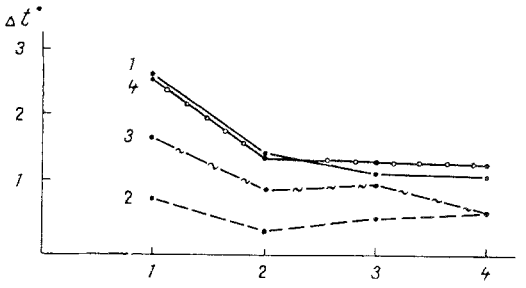

Fig. 3

Fig. 2. Effect of TSHRH and its analogs on development of tolerance to ethanol by the revolving rod test. Abscissa, duration of experiment (in days), ordinate, number of falls. 1) Control, 2) TSHRH, 3) TSHRH-2, 4) TSHRH-3.

Fig. 3. Effect of TSHRH and its analogs on development of tolerance to hypothermic effect of ethanol. Ordinate, Δt°C. Remainder of legend as in Fig. 2.

action of TSHRH and of some of its analogs. The possibility of dissociation of behavioral and hormonal properties in the TSHRH molecule is accepted by some workers [1, 4, 5, 7]. Moreover, a synthetic analog of TSHRH with high psychotropic activity, practically free from any hormonal properties of any kind, is now known. However, within the group of preparations which we tested, correlation was found between hormonal activity and ability to counteract the fall of rectal temperature caused by injection of ethanol. All compounds, irrespective of their specific properties, were ineffective in preventing the action of ethanol revealed by the revolving rod test, nor did they affect the development of tolerance. The degree of participation of the hormonal component of TSHRH and of its analogs in the mechanism of their neurotropic action thus varies when different effects are studied, probably because of the involvement of nonidentical mechanisms of realization at the level of the CNS or the endocrine system proper. Since attempts have been made to use TSHRH in psychiatric and drug addiction practice [8, 9], an oriented search for TSHRH analogs with selective psychotropic and "antialcoholic" properties would seem to be promising.

LITERATURE CITED

1. V. N. Babichev, V. M. Samsonova, M. G. Airapet'yants, et al., Probl. Éndokrinol., No. 4, 35 (1981).
2. A. P. Smirnova, G. I. Bushueva, V. I. Gudoshnikov, et al., in: Prospects for Bio-Organic Chemistry in the Creation of New Therapeutic Preparations [in Russian], Riga (1982), p. 81.
3. G. R. Breese, G. M. Cott, B. C. Cooper, et al., J. Pharmacol. Exp. Therm., 195, 11 (1975).
4. G. M. Cott, G. R. Breese, B. C. Cooper, et al., J. Pharmacol. Exp. Therm., 196, 594 (1976).
5. N. Fukuda, O. Nishimura, M. Shikata, et al., Chem. Pharm. Bull., 28, 1667 (1980).
6. J. W. Holaday, L. F. Tseng, H. H. Loh, et al., Life Sci., 22, 1537 (1978).
7. H. Kruse, in: Depressive Disorders: Symposium, Stuttgart (1978), p. 237.
8. M. Linoila, M. Y. Mattila, P. Karhunen, et al., Eur. J. Pharmacol., 21, 27 (1981).
9. H. P. Vogel, O. Benkert, R. Illig, et al., Acta Psychiat. Scand., 56, 223 (1977).