SYNTHESIS AND STUDY OF THE INFLUENCE OF CERTAIN PRODUCTS OF SEROTONIN METABOLISM, β-CARBOLINES AND RELATED COMPOUNDS, ON THE VOLUNTARY CONSUMPTION OF ALCOHOL IN ANIMALS

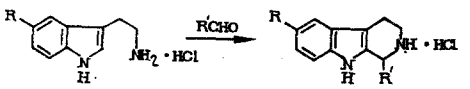
Yu. V. Burov, V. A. Zagorevskii,
V. N. Zhukov, N. N. Novikova,
and I. D. Silenko

UDC 616.89-008.441.13-02:577.175.823]-092.9

It is suggested that certain natural serotonin metabolites [1], as well as their cyclic analogs, representing compounds of the group of β-carbolines, may play a substantial role in processes of formation and manifestation of alcohol dependence, since they intervene in the regulation of alcohol consumption [2, 3], being products of *in vivo* cyclization of serotonin and its metabolites with acetaldehyde — a metabolite of ethanol [4]. The incorporation of these compounds into the mechanism of formation of alcohol dependence may be determined by their ability to intervene in certain neurochemical processes [4], in particular, the serotoninergic processes. Moreover, the high affinity for the benzodiazepine receptor [5] suggests possible intervention of the compounds under consideration in the emotional processes associated with the attraction to alcohol.

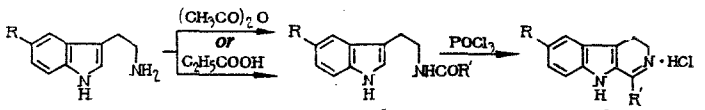
In connection with the aforementioned, the aim of this work was to synthesize model compounds (analogs of natural serotonin metabolites), cyclization products of serotonin and mexamine with acetaldehyde, as well as related compounds (Ia-*l*, IIa-d, IIIa-d), and to study their influence on the voluntary consumption of alcohol in an experiment on animals to establish the possible dependence of their pharmacological activity on their chemical structure.

The syntheses were performed according to the schemes



I a-l

Ia: R = OCH_3, R′ = H; *b*: R = OCH_3, R′ = CH_3; *c*: R = OCH_3, R′ = C_2H_5; d: R = OCH_3, R′ = C_3H_7-n; *e*: R = OCH_3, R′ = $CH(CH_3)_2$; *f*: R = OCH_3, R′ = C_4H_9-n; *g*: R = OCH_3, R′ = C_6H_{13}-n; *h*: R = OH, R′ = CH_3; *i*: R = OH, R′ = C_2H_5; *j*: R = OH, R′ = C_3H_7; *k*: R = OH, R′ = C_4H_9-n; *l*: R = OH, R′ = C_6H_{13}-n.



II a-d *III a-d*

II, IIIa: R = H, R′ = CH_3; *b*: R = H, R′ = C_2H_5 *c*: R = OCH_3, R′ = CH_3; *d*: R = OCH_3, R′ = C_2H_5.

EXPERIMENTAL PHARMACOLOGY

The experiments were conducted on 160 noninbred mice (males), weighing 26-36 g, under conditions of free selection between a 10% solution of ethanol and water for 1-6 months. The animals were kept individually in cages with dimensions 4 × 14 × 5 cm with two graduated water dispensers, filled round-the-clock with alcohol and water. Mice whose daily alcohol consumption was 5-11 g/kg, converted to pure ethanol, were selected for the experiments. The daily consumption of alcohol and water five days before the beginning of administration

Institute of Pharmacology, Academy of Sciences of the USSR, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 17, No. 8, pp. 936-940, August, 1983. Original article submitted October 26, 1982.

0091-150X/83/1708-0559$07.50

TABLE 1. Influence of Some Metabolites of Serotonin, β-Carbolines, and Their Analogs on Voluntary Consumption of a 10% Ethanol Solution by Mice

Compound	LD_{50}, mg/kg	Period of alcohol treatment, mos.	Dose, mg/kg	Average consumption calculated on the basis of pure ethanol			Average daily water consumption, incl. water of the ethanol solution		
				background 5 days, g/kg	% of background in 5 days of admin. of sub.	% of background in 5 days of deprivation	background 5 days, g/kg	% of background in 5 days of admin. of sub.	% of background in 5 days of deprivation
Ia	500	6	50	6,8 (8,8)	82 (77)	97 (77)	119 (139)	99 (96)	95 (94)
Ib	310	2,5	20	5,6 (9,6)	64* (116)	63* (155)	213 (221)	101 (149)*	108 (148)
Ic	320	1	32	5,0 (8,3)	91 (80)	205 (137)	156 (142)	70 (76)	108 (113)
Id	260	6	26	8,0 (8,8)	56 (77)	62 (77)	144 (139)	111 (96)	109 (94)
Ie	320	1	32	5,4 (8,3)	52* (80)	108 (137)	149 (142)	92 (76)	128 (113)
If	330	1	32	9,1 (8,3)	65 (80)	111 (137)	163 (142)	60 (76)	98 (113)
Ig	170	6	17	6,0 (8,8)	56* (77)	73* (77)	170 (139)	96 (96)	83 (94)
Ih	750	2,5	40	6,7 (9,6)	59* (116)	62 (155)	232 (221)	111* (149)*	109 (148)
Ii	430	1	43	8,8 (8,3)	77 (80)	130 (137)	150 (142)	100 (76)	191 (113)
Ij	500	6	50	8,0 (8,8)	52* (77)	72 (77)	146 (139)	111* (96)	120 (94)
Ik	500	1	50	7,8 (8,3)	54* (80)	75 (137)	163 (142)	67 (76)	103 (113)
Il	180	6	18	8,0 (8,8)	66* (77)	48* (77)	149 (139)	99 (96)	89 (94)
IIb	900	1	90	6,4 (8,3)	102 (80)	213* (137)	156 (142)	68 (76)	132 (113)
IIc	1375	3	150	11,3 (7,2)	81* (58)	78 (114)	179 (175)	94 (89)	117* (110)*
IId	850	1	85	7,1 (8,3)	51 (80)	117 (137)	183 (142)	96 (76)	125 (113)
IIIc	10,5	1	1	7,4 (7,6)	118 (134)	73 (75)	127 (172)	95* (123)	90 (106)

Note. Here and in Table 2, the indices in the control are cited in parentheses; a statistically significant difference at $P < 0.05$ (relative to the background level) is marked by an asterisk.

of the substances studied (background), after five days of their administration, and five days before the end of the administration (deprivation) of the substances studied, was determined. Each experimental group of animals had its corresponding control group of mice with the same periods of contact with alcohol, which received intraperitoneal injections of distilled water in volumes equal to the volumes of the solutions of the substances studied to be introduced. The substances were introduced twice a day for a period of five days (at 1000 and 1600 h) intraperitoneally in doses of 1/10 LD_{50}. Experiments conducted on 36 noninbred rats (males) differed from the experiments on mice in the period of voluntary consumption of alcohol (more than eight months), its concentration (15% ethanol solution), duration of the background period and deprivation period (one week each), and duration of administration of the substances (for two weeks). LD_{50} of the synthesized compounds was determined by the method of Lichfield and Wilcoxon [7]. Statistical treatment of the data on the consumption of liquids was performed by the Meddis method [7].

The results of our experiments showed (Table 1) that under the influence of compounds belonging to the group of 1,2,3,4-tetrahydrocarbolines, the level of voluntary consumption of alcohol is lowered. The greatest activity in this respect was exhibited by model compounds — cyclic derivatives of serotonin (compound Ih) and mexamine (compound Ib). The indicated compounds reduce the alcohol consumption by more than 50% when they are administered and by more than 90% after their deprivation in comparison with the control; they reduce water consumption simultaneously with this.

Homologs of Ih (Ii, Ij, Ik, and Il) inhibit alcohol consumption less than compound Ih. Moreover, their activity disappears when the aliphatic chain is lengthened by only one carbon atom and increases in the presence of three to four carbon atoms in the indicated position. A further lengthening of the chain (Il) again leads to a substantial weakening of the effect against a background of administration of the substance; however, in this case an aftereffect begins to appear: a decrease in alcohol consumption after the introduction of the substance. It should be noted that the strength of the inhibiting action on alcohol consumption among the compounds under consideration is not associated with the degree of their toxicity: The more active substances (Ij, Ik) have lower toxicity than the less active substances (Il, Ii).

Homologs of compound Ib also proved less active with respect to inhibition of the voluntary consumption of alcohol than compound Ib. In this series there is no activity both for the lower homolog Ia and when the aliphatic chain is lengthened to 2, 3, and 4 carbon atoms; activity begins to appear in the presence of an aliphatic chain with six carbon atoms. Compound Ie, possessing an isopropyl group in the 1-position, proved active. On the whole, analogs Ib are less toxic than analogs Ih.

Melatonin (IIc) exhibits an ability to increase the average daily ethanol consumption against a background of its use (in comparison with the control). After its administration, a certain increase in the water consumption is noted. Lengthening the aliphatic chain of

TABLE 2. Influence of Substances Ih and Ib on the Consumption of a 15% Ethanol Solution among Rats That Had Voluntarily Consumed It for More Than Eight Months

Compound	Initial (background) nature of behavior toward alcohol	Dose, mg/kg	Average daily consumption of pure ethanol						Average daily consumption of water, including water of ethanol solution						
			background, g/kg (one week)	administration of substance				deprivation for 1 week		background, g/kg (one week)	administration of substance				deprivation for 1 week	
				1 week		2 weeks					1 week		2 weeks			
				% of background	difference between indices in exp. and control, %	% of background	difference between indices in exp. and control, %	% of background	difference between indices in exp. and control, %		% of background	difference between indices in exp. and control, %	% of background	difference between indices in exp. and control, %	% of background	difference between indices in exp. and control, %
Ib	"Light-drinking"	20	4,3 (3,2)	128* (89)	+39	97 (92)	+5	152* (116)	+36	55 (57)	106 (100)	+6	105 (108)	—3	141* (111)	+50
	"Heavy-drinking"	20	11,8 (7,5)	80* (103)	23	60* (90)	—30	70* (92)	—22	74,5 (60,5)	88 (98)	—10	88 (102)	—14	102 (104)	—2
Ih	"Light-drinking"	75	3 (3,2)	82* (89)	—7	105 (92)	+13	151* (116)	+35	40,5 (57)	112 (100)	+12	111 (108)	+3	132* (111)	+21
	"Heavy drinking"	75	8,9 (7,5)	74* (103)	—29	63* (90)	—27	83* (92)	—9	59 (60,5)	89* (98)	—9	77* (102)	—25	91 (104)	—13

melatonin by one carbon atom (IId) eliminates the indicated ability. The analog of IIc without a methoxy group in the 5-position and with an acyl radical lengthened by one carbon atom (IIb) induces an increase in the ethanol consumption after the administration of this substance is stopped.

With respect to influence on water consumption, among the analogs of Ib and Ih, relatively low activity was exhibited by compound Ij, which increased the water consumption when it was administered. The nature of the changes in the water consumption under the influence of the remaining analogs considered proved statistically insignificant.

Taking into account the fact that among the investigated compounds substances Ib and Ih decrease the alcohol consumption by mice to the greatest degree, we studied their influence on the alcohol motivation in rats that had voluntarily consumed a 15% ethanol solution for more than eight months, i.e., at the periods when there may be an established dependence on ethanol under these conditions [8]. The results of our experiments showed (Table 2) that the nature of the changes in alcohol consumption among rats under the indicated conditions depends on the initial level of alcohol motivation. In rats that had initially consumed relatively large amounts of ethanol (from 7.5 to 11.8 g/kg per day – "heavy drinking" animals), both compounds decreased the alcohol consumption both while they are being administered and after deprivation. Among rats that had been consuming relatively small amounts of ethanol (from 3 to 4.3 g/kg per day – "light-drinking" animals), under the influence of Ib the alcohol consumption increases while it is being administered, especially in the first week of administration. Under the influence of Ih, the alcohol consumption decreases negligibly during this period. After cessation of the administration of both compounds under consideration, the alcohol consumption increases among the "light-drinking" animals.

Thus, investigations conducted on mice provide evidence that the greatest suppressing effect on alcohol consumption is given by cyclic derivatives of serotonin and mexamine. All the compounds with a modified structure studied proved less active. Melatonin increases the alcohol consumption while it is being administered; after deprivation of melatonin there is a weakening of the alcohol motivation. Among compounds differing from melatonin by the absence of the methoxy group and the presence of a longer aliphatic chain, an aftereffect is detected: an increase in alcohol consumption after the substance has ceased to be administered. A compound differing from the latter by the presence of a methoxy group causes a decrease in alcohol consumption while this substance is being administered and after its administration has ceased. An analogous effect is given by a cyclic analog of melatonin – 1-methyl-6-methoxy-3,4-dihydro-β-carboline (IIIc).

The data obtained in experiments on rats show that cyclic derivatives of serotonin (Ih) and mexamine (Ib) intervene in the regulation of the voluntary consumption of alcohol; in this case their effect depends on the initial level of alcohol motivation under conditions of a physical dependence on ethanol.

EXPERIMENTAL CHEMISTRY

1-Methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline Hydrochloride (Ib). To a solution of 53.3 g (0.235 mole) mexamine hydrochloride in 1.8 liters of acetate buffer (pH 4.7), 14.3 g

TABLE 3. Properties of Compounds I-III

Compound	Yield, %	mp, °C	Found, %				Gross formula	Calculated, %			
			C	H	N	Cl		C	H	N	Cl
Ia	87	262–3	...	...	11,63	14,97	$C_{12}H_{15}ClN_2O$	...	...	11,78	15,27
If	48	246–7	...	...	9,45	12,05	$C_{16}H_{23}ClN_2O$	...	...	9,5	12,03
Ig	21	246–7	...	...	8,69	10,92	$C_{19}H_{27}ClN_2O$	...	...	8,67	10,98
Ij	49	233–4	...	...	10,63	14,50	$C_{13}H_{17}ClN_2O$	...	...	11,08	14,03
Ik	38	198–9	...	...	9,74	12,80	$C_{18}H_{21}ClN_2O$	...	...	9,98	12,63
Il	65	185–6	...	...	8,94	11,54	$C_{17}H_{25}ClN_2O$	...	...	9,07	11,48
IIb	87	83–4	72,02	7,68	13,06	...	$C_{15}H_{18}N_2O$	72,19	7,46	12,95	...
IId	78	90–1	68,21	7,5	11,62	...	$C_{14}H_{19}N_2O$	68,26	7,36	11,37	...
IIIa	61	247–8	...	...	...	15,98	$C_{12}H_{13}ClN_2O$	...	...	...	16,07
IIIb	88	245–6	66,22	6,26	11,95	15,5	$C_{13}H_{15}ClN_2O$	66,5	6,4	11,9	15,1
IIIc	63	247–8,5	...	...	11,32	13,9	$C_{12}H_{11}ClN_2O$	...	...	11,17	14,14
IIId	47	241–1,5	63,31	6,55	10,73	13,7	$C_{14}H_{17}ClN_2O$	63,51	6,47	10,58	13,39

Note. The base Ii, mp 179.5-180.5 °C. Found, %: C 72.35; H 7.48; N 13.01. $C_{13}H_{16}N_2O$. Calculated, %: C 72.18; H 7.45; N 12.95. The base Il, mp 165.5-167 °C. Found, %: C 75.25; H 8.85; N 10.28 $C_{14}H_{18}N_2O$. Calculated, %: C 74.95; H 8.88; N 10.28.

(0.325 mole) acetaldehyde is added, and the mixture left for two days at room temperature. The reaction mass is alkalinized with a 30% solution of sodium hydroxide, extracted with chloroform, and dried with magnesium sulfate. The chloroform is distilled off, the residue dissolved in alcohol, a solution of hydrogen in alcohol added, and 50.2 g (84%) of the hydrochloride Ib is obtained. The hydrochlorides Ia, c-h are produced analogously.

1-Propyl-6-hydroxyl-1,2,3,4-tetrahydro-β-carboline Hydrochloride (Ij). To a solution of 1 g (3 mmoles) serotonin adipate in 20 ml of acetate buffer (pH 4.7) and 5 ml of alcohol, 0.4 g (0.55 mole) butyraldehyde was added. The reaction mass was heated on a water bath for 4 h, a solution of potash was added, extracted with methylene chloride, the solvent distilled off, and 0.6 g (74%) of the base Ij obtained; it was converted to the hydrochloride just like the preceding compound. The hydrochlorides Ii, Ik, and Iℓ were produced analogously.

Compounds Ia, If, Ig, Ii, Ik, Iℓ, IId, and IIId have not been described in the literature (Table 3). Substances I and II were produced according to the well-known procedures [9-11]; moreover, according to the literature data, substance IIb was obtained in the form of an oil, while substances Ia and IIIa-c were characterized only in the form of the bases.

LITERATURE CITED

1. P. K. Rudeen and S. K. Symmes, Pharmacol. Biochem. Behav., 14, 143-147 (1981).
2. I. Geller, R. Purdy, and J. H. Merrit, Ann. N. Y. Acad. Sci., 215, 54-59 (1973).
3. R. D. Myers and M. M. Olbinger, Drug Alcohol Depend., 2, 469-483 (1981).
4. M. M. Airaksinen and I. Kari, Med. Biol., 59, 21-34 (1981).
5. C. Braestrup and M. Nielsen, Trends Pharmacol. Sci., 1, 424-427 (1980).
6. M. L. Belen'kii, Elements of the Quantitative Evaluation of the Pharmacological Effect [in Russian], Second Edition, Leningrad (1963).
7. R. Meddis, Br. J. Psychol., 66, 225-227 (1975).
8. Yu. V. Burov, V. N. Zhukov, and A. B. Kampov-Polevoi, Methodological Recommendations for the Experimental (Pharmacological) Study of Preparations Proposed for Clinical Testing as Agents for the Prophylaxis and Therapy of Alcoholism [in Russian], Moscow (1980).
9. G. Blasko, K. Honty, and L. Novak, Acta Chim. Acad. Sci. Hung., 99, 35-41 (1979).
10. E. Spath and E. Leberer, Chem. Ber., 63, 2162 (1930).
11. M. F. Petrova, N. S. Kaverina, and G. P. Men'shikov, Zh. Obshch. Khim., 33, 1333 (1963).