MOLECULAR-BIOLOGICAL PROBLEMS IN DRUG CREATION AND THE STUDY OF THE MECHANISM OF THEIR ACTION

AMINO ALCOHOL DERIVATIVES: EFFECTS OF ALCOHOL METABOLISM ENZYMES

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A number of amino alcohols have been synthesized as part of the search for effective chemotherapeutic agents for alcoholism; their effects on the activity of enzymes involved in alcohol metabolism — alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) — have been investigated.

Information is currently available on the effects of various chemical agents on ADH and ALDH [1]. However, of greatest interest are analogues of the substrate which, because of their structural similarity to ethanol and acetaldehyde, are bound to the active site and exert a specific effect on ADH and ALDH. It could be assumed that specific effectors of ADH and ALDH may have promise as chemotherapeutic agents in the treatment of various forms of alcoholism and alcohol toxicity.

We elected to study derivatives of aminopropanols, the effects of which on ADH and ALDH have not been investigated to date.

Ethoxypropanols, i.e., 3-ethoxy-1-(N-benzylamino)-propanol-2 (II) and 3-ethoxy-1-(N-p-tolylamino)-propanol-2 (III), were prepared by reaction of 3-ethoxy-1-chloropopanol-2 (I) with the respective amines:

$$C_2H_5OCH_2CH(OH)CH_2Cl + 2NH_2R = C_2H_5OCH_2CH(OH)CH_2NHR + NH_2R \cdot HCl$$
$$R = CH_2C_6H_5 \text{ (II)};\ C_6H_4CH_3 \text{ (III)}.$$

The synthesis of aliphatic-aromatic aminopropanols (V, VII, and IX-XI) and a benzoyl derivative (XII) was achieved by a three-step process.

Using acetophenone in the Mannich reaction according to conditions previously determined [2-5], the corresponding aminoketones (IV, VI, VIII) were obtained; subsequent reduction of the keto groups or reaction with Grignard reagents resulted in their transformation into the corresponding secondary (V, VII, IX) or tertiary (X, XI) alcohols (6, 7].

$$C_6H_5COCH_3 + CH_2O + \left\{\begin{array}{lll} HN(CH_3)_2 & \longrightarrow C_6H_5CO(CH_2)_2N(CH_3)_2 \text{ (IV)} \xrightarrow{H} & C_6H_5CH(OH)(CH_2)_2N(CH_3)_2 \text{ (V)} \\ HNC_5H_{10} & \longrightarrow C_6H_5CO(CH_2)_2NC_5H_{10} \text{ (VI)} \xrightarrow{H} & C_6H_5CH(OH)(CH_2)_2NC_5H_{10} \text{ (VII)} \\ HNC_4H_8O & \longrightarrow C_6H_5CO(CH_2)_2NC_4H_8O \text{ (VIII)} \xrightarrow{H} & C_6H_5CH(OH)(CH_2)_2NC_4H_8O \text{ (IX)} \end{array}\right.$$

$$IV + RMgX \longrightarrow C_6H_5C(OH)(CH_2)_2N(CH_3)_2$$
$$\Big|$$
$$R_1$$
$$R_1 = CH_2C_6H_5 \text{ (X)};\ C_2H_5 \text{ (XI)}$$

Benzoylation with 1-dimethylamino-3-phenylpentanol led to the corresponding ester (XII) [8].

$$XI + C_6H_5COCl \longrightarrow C_6H_5-\underset{\displaystyle C_2H_5}{\overset{\displaystyle OCOC_6H_5}{\overset{|}{\underset{|}{C}}}}-CH_2CH_2N(CH_3)_2$$

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Taking into consideration the unique behavior of aminoketones during catalytic hydrogenation noted earlier [9], the secondary aminopropanols (V, VII, IX) were obtained in 60-70% yield using the modified method employing sodium borohydride.

All the aminopropanols were obtained as hydrochlorides: stable, colorless crystals highly soluble in water. The constants of the synthesized compounds, both the base and the hydrochlorides, were in agreement with the information in the literature.

Structures of the resultant amino alcohols were confirmed by IR spectroscopy; the spectra showed a wide absorption band in the 3420-3470 cm^{-1} region, corresponding to stretching vibrations of the bound HO group. The IR spectra of the hydrochloride of the benzoyl derivative, XII, lacked such absorption bands, but showed distinct intense absorption bands at 1720-1735 cm^{-1} which are characteristic of the COOR group. Absorption bands at 1580 and 1615 cm^{-1} were ascribed to vibrations of the aromatic ring, at 1270 cm^{-1} to vibration of the —C—O bond, and at 2550 cm^{-1} to the N^+H bond.

EXPERIMENTAL (CHEMICAL)

The IR spectra were obtained with a UR-20 spectrophotometer (East Germany), using amino alcohol bases as a liquid film, and the hydrochlorides as pastes in vaseline oil.

3-Ethoxy-1-(N-benzylamino)-2-propanol (II). A mixture of 0.1 moles I [10] and 0.2 moles benzylamine was heated at 80-85°C for 4 h. After cooling, the benzylamine hydrochloride was removed by filtration and washed several times with dry ether; the ether was removed by distillation and the residue was redistilled. Yield: 75%. bp 52°C at 0.4 mm Hg; n_D^{20} 1.5032; d_4^{20} 0.9844. Found, %: C 68.39; H 9.28; N 6.65. $C_{12}H_{19}NO_2$. Calculated, %: C 68.87; H 9.15; N 6.69. IR spectrum, cm^{-1}: $\nu_{C=C}$ 1610, 1460; ν_{OH} 3390; ν_{C-O-C} 1120.

3-Ethoxy-1-(N-p-tolylamino)-2-propanol (III) was obtained in an analogous manner with an 85% yield. bp 90-92°C at 10 mm Hg; n_D^{20} 1.5039; d_4^{20} 1.0201. Found, %: C 69.39; H 9.28; N 6.65. $C_{12}H_{19}NO_2$. Calculated, %: C 68.87; H 9.15; N 6.69. IR spectrum, cm^{-1}: $\nu_{C=C}$ 1615, 1475; ν_{OH} 3410; ν_{C-O-C} 1135.

Preparation of Amino Alcohol Hydrochlorides. After cooling to 0°C a 0.1 M solution of II or III in 50 ml absolute ether, the solution was exposed to dry hydrogen chloride, and the residue was obtained by filtration and washed several times with absolute ether, the hydrochloride was obtained in 90% yield. mp 189-191°C (from acetone with decomposition). Found, %: N 5.75; Cl 14.16. $C_{12}H_{19}NO_2 \cdot HCl$. Calculated, %: N 5.68; Cl 14.37. IR spectrum, cm^{-1}: $\nu_{NH_2}^{+}$ 2570.

The hydrochloride of III was obtained in an analogous manner with 80% yield. mp 201-202°C (from acetone with decomposition). Found, %: N 5.28; Cl 14.46. $C_{12}H_{19}NO_2 \cdot HCl$. Calculated, %: N 5.68; Cl 14.37. IR spectrum, cm^{-1}: $\nu_{NH_2}^{+}$ 2575.

Preparation of 3-Amino-1-phenylpropanols.* A mixture consisting of 0.02 moles beta-aminopropiophenone, 0.02 moles potassium hydroxide, and 0.04 moles sodium borohydride in 60 ml methanol and 15 ml water was kept at 20°C for 24 h. The precipitated potassium chloride was removed, aqueous methanol was eliminated by distillation, and the residue was treated with 18% hydrochloric acid. Neutral products were extracted with ether and the aqueous layer was made alkaline with sodium carbonate and extracted several times with ether. The pooled ether extracts were washed with a small amount of water, separated from the aqueous layer, and dried with magnesium sulfate. The ether was removed by distillation and the product was redistilled under vacuum.

The constants of the aminopropanols V, VII, and IX prepared by the above methods were in agreement with those prepared by catalytic hydrogenation [2, 3, 11].

The previously undescribed VII hydrochloride was prepared in the usual manner, mp 134-136°C. Found, %: N 5.43; 5.30. $C_{14}H_{21}NO \cdot HCl$. Calculated, %: N 5.48.

EXPERIMENTAL (BIOCHEMICAL)

Use was made of a commercial crystalline ADH from equine liver (Reanal, Hungary). Immediately before use the enzyme was desalted on a column of Sephadex G-25, medium size. The protein concentration was determined at 280 nm, using a molar extinction coefficient for ADH of $E_{1\,cm}^{0.1\%} = 0.46$ [12]. The β-NAD^+ used was also a Reanal product (Hungary). Prior to use, the

*Jointly with E. Ya. Borisova.

TABLE 1. Interaction of Amino Alcohol Derivatives with ADH and ALDH

Compound	Name	Formula	$I_{50}, \cdot 10^{-3}$ M	
			ADH	ALDH
I	3-Ethoxy-1-chloro-propanol-2	$C_2H_5OCH_2CHCH_2Cl$ (OH)	—	—
II	3-Ethoxy-1-(N-benzylamino)-propanol-2 HCl	$C_2H_5OCH_2CHCH_2NHCH_2C_6H_5HCl$ (OH)	0,42	>20
III	3-Ethoxy-1-(N-p-tolylamino)-2-propane HCl	$C_2H_5OCH_2CHCH_2NHC_6H_4CH_3 \cdot HCl$ (OH)	5,3	>20
IV	β-Dimethylaminopropiophenone HCl	$C_6H_5COCH_2CH_2N(CH_3)_2 \cdot HCl$	>20	2,5
V	1-Phenyl-3-dimethylamino-1-propanol HCl	$C_6H_5CHCH_2CH_2N(CH_3)_2 \cdot HCl$ (OH)	>20	>20
VI	β-Piperidylpropiophenone HCl	$C_6H_5COCH_2CH_2NC_5H_{10} \cdot HCl$	16	1
VII	1-Phenyl-3-(N-piperidyl)-1-propanol HCl	$C_6H_5CHCH_2CH_2NC_5H_{10} \cdot HCl$ (OH)	>20	>20
VIII	β-(N-morpholino)-propiophenone HCl	$C_6H_5COCH_2CH_2NC_4H_8O \cdot HCl$	>20	>20
IX	1-Phenyl-3-(N-morpholino)-1-propanol HCl	$C_6H_5CHCH_2CH_2NC_4H_8O \cdot HCl$ (OH)	>20	>20
X	4-Dimethylamino-1,2-diphenyl-2-butanol HCl	$C_6H_5CCH_2CH_2N(CH_3)_2 \cdot HCl$ (OH, $CH_2C_6H_5$)	>20	>20
XI	1-Dimethylamine-3-phenyl-3-pentanol HCl	$C_6H_5CCH_2CH_2N(CH_3)_2 \cdot HCl$ (OH, C_2H_5)	—	—
			$A_{50}, \cdot 10^{-3}$ M	
XII	1-Dimethylamino-β-phenyl-3-benzoylpentane HCl	$C_6H_5CCH_2CH_2N(CH_3)_2 \cdot HCl$ ($OCOC_6H_5$, C_2H_5)	2,0	0,8

acetaldehyde and ethanol were distilled. The Sephadex products used were G-25 and G-200 (Pharmacia, Sweden).

ALDH was isolated from rat liver as previously described [13]. The enzyme preparation was relatively stable for a period of one month. The specific activity was, on the average, $2 \cdot 10^{-5}$ M NAD·H mg^{-1} protein min^{-1}.

Changes in Enzymatic Activity. Kinetic measurements were conducted with a rate analyzer, 4200 A LKB (Sweden), on a scale of 0-0.05 OU/20 cm with an LKB 6500 recorder at 35°C. Spectrophotometric measurements were made with a Hitachi (Japan) 200-20 double-beam spectrophotometer. The initial rate of stationary reduction of NAD^+ was determined from the change in optical density at 340 nm, using the coefficient for molar extinction for NAD·H of $E_m = 6.22 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ [14]. Reactant concentrations are indicated in the figures.

The nature of inhibition was determined from the dependence of the initial rate of the stationary reaction on NAD^+ concentration in the presence of constant concentrations of the second substrate and inhibitors, in accordance with the Lineweaver–Burk criteria [15]. Values of I_{50} or A_{50} were determined from the dependence of the initial rate of NAD^+ reduction on the concentrations of inhibitors or activators in the presence of constant saturating concentrations of the enzyme and substrate. All the studies on the enzymatic activity of ADH were conducted in 0.05 M sodium phosphate buffer, pH 7.2, with the addition of 0.02 M semicarbazide HCl. Experiments with ALDH were conducted in 9.2 M glycine buffer, pH 9.2.

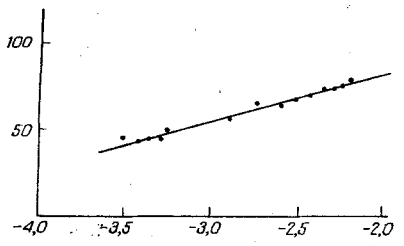

Fig. 1

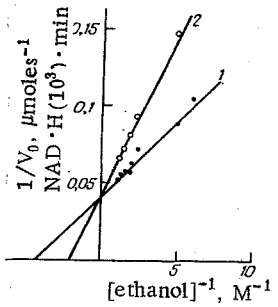

Fig. 2

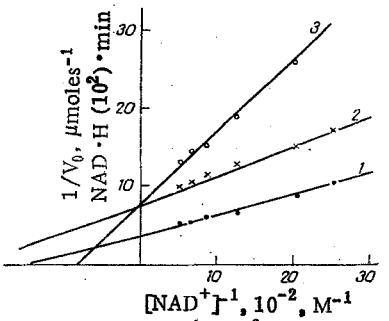

Fig. 3

Fig. 1. Determination of I_{50} for II HCl (ADH). Concentrations: $3.5 \cdot 10^{-8}$ M ADH, $1.0 \cdot 10^{-2}$ methanol, $1.0 \cdot 10^{-3}$ M NAD^+. Ordinate: percent inhibition; abscissa: log I.

Fig. 2. Inhibition of ADH by III HCl with respect to ethanol. Concentrations: $3.5 \cdot 10^{-8}$ M ADH, $1.0 \cdot 10^{-3}$ M NAD^+. 1) Without inhibitor; 2) $1.0 \cdot 10^{-3}$ M III HCl.

Fig. 3. Inhibition of ADH by III HCl with respect to NAD^+. Concentrations: $3.5 \cdot 10^{-8}$ M ADH, $1.0 \cdot 10^{-2}$ M ethanol. 1) Without inhibitor; 2) $1.0 \cdot 10^{-3}$ M III HCl; 3) $2.0 \cdot 10^{-3}$ M III HCl.

RESULTS AND DISCUSSION

Interaction of Amino Alcohol Derivatives with Horse Liver ADH and Rat Liver ALDH

The data in Table 1 illustrate the various effects encountered on exposure of ADH to the amino alcohol derivatives. Compound II showed the most pronounced inhibition: $I_{50} = 4.2 \cdot 10^{-4}$ M. This amino alcohol has an aromatic ring bound to nitrogen via a methylene group. In III, an aminopropanol, the aryl residue is bound directly to nitrogen and the value for I_{50} is increased almost by an order of magnitude. The presence of phenyl groups in the main chain of the aliphatic-aromatic alcohols has a negative effect on their reactivity with the enzyme (compounds V, VII, VIII, IX, X). An unexpected activation of ADH was observed with the ester XII: $A_{50} = 2 \cdot 10^{-3}$ M.

In the case of ALDH only two of the compounds (IV and VI) were found to be weak inhibitors. However, A_{50} for XII was much lower with ALDH than the ADM and was equal to $8 \cdot 10^{-4}$ M. We have not found any mention in the literature of such a powerful activator for ADH and ALDH. We did not perform a detailed analysis of the mechanism of action of this compound; however, we did observe that in the absence of the natural substrate, ethanol, NAD^+ is not reduced.

Figure 1 presents typical plots of the dependence of the initial rate of the stationary ethanol oxidation reaction on the inhibitor concentration, using semilogarithmic coordinates.

We studied the character of inhibition in the Lineweaver–Burk coordinates [15] for some of the amino alcohols with respect to the coenzyme and the substrate. Figures 2 and 3 show that in the case of III the inhibition is competitive with respect to ethanol and is of the mixed type with respect to NAD^+.

Our results lead to the conclusions that inhibitors and activators of the enzymes involved in ethanol metabolism can be found among the amino alcohols and amino esters, which should be investigated on various animal models of alcohol intoxication.

Furthermore, our results point to the advantages of searching for modulators of enzymes involved in ethanol metabolism among ethanol congeners and related compounds.

LITERATURE CITED

1. E. I. Shchors and R. E. Libinzon, Khim.-Farm. Zh., No. 7, 3-14 (1978).
2. C. Mannich and G. Heilner, Chem. Ber., 55, 356 (1922).
3. C. Mannich and D. Lammering, Chem. Ber., 55, 3510 (1972).
4. R. Harradance, Proc. Roy. Soc. London, Ser. B, 72, 233 (1938).
5. E. M. Cherkasova, Inventor's Certificate 128467 (SSSR); Byull. Izobret., No. 10 (1960).
6. I. N. Nazarov and E. M. Cherkasova, Zh. Obshch. Khim., 25, 1541 (1955).
7. I. N. Nazarov and E. M. Cherkasova, Zh. Obshch. Khim., 25, 1539 (1955).
8. I. N. Nazarov and E. M. Cherkasova, Zh. Obshch. Khim., 25, 1544 (1955).
9. E. M. Cherkasova, Zh. Obshch. Khim., 2846 (1960).
10. H. Flores Caliardo and C. D. Pollard, J. Org. Chem., 12, 831 (1947).

11. J. H. Burekhalter and S. M. Johnson, J. Am. Chem. Soc., 73, 4827 (1951).
12. S. Libor, P. Elodi, and Z. Nagy, Biochim. Biophys. Acta, 110, 484-490 (1965).
13. E. I. Shchors, Khim.-Farm. Zh., No. 2, 19-24 (1979).
14. J. M. Siegel and G. A. Montgomery, Arch. Biochem., 82, 288-293 (1959).
15. H. Lineweaver and D. Burk, J. Am. Chem. Soc., 56, 658-666 (1934).

COMPARATIVE STUDIES ON THE PHARMACOKINETICS OF THE ANTIHISTAMINIC FENKAROL AND ITS o-TOLYL CONGENER

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Earlier reports have dealt with the pharmacokinetics of the original Soviet antihistaminic (antiallergic) fenkarol (I; quinuclidyl-3-diphenylcarbinol HCl) [1, 2], which belongs to a class of chemicals newly identified as having antihistaminic properties; it is currently being manufactured commercially by the pharmaceutical industry. Pharmacologic testing of other derivatives of quinuclidyl-(diaryl, heteryl)carbinol (congeners of I) revealed compounds which also possess antihistaminic properties and differ in the duration of action [3].

One of these compounds was quinuclidyl-3-di(o-tolyl)carbinol (II).

It appeared of interest to correlate the duration of action of these agents to their pharmacokinetics. Comparisons were made of single and multiple administrations. The study utilized drugs labeled in the 2 and 3 positions of the quinuclidyl nucleus with ^3H [4].

EXPERIMENTAL

The studies were conducted on 100-120 g albino male Wistar rats. In series I experiments the animals received a single administration of the agent with the 2 and 3 positions of the quinuclidyl nucleus labeled with ^3H, having a specific activity of 29.7 MCi/g. The radioactivity for the entire animal at a dose of 50 mg/kg (1/20th LD_{50}), which had a pronounced pharmacologic effect, was equal to 49.7 μCi. II was given intragastrically as a 1% homogeneous suspension in 1% carboxymethyl cellulose. Immediately after drug administration the animals were primed with water (5 ml 0.45% sodium chloride per 100 g body weight) and placed into metabolic cages. Stool and urine production were determined after 1, 3, 6, 12, 24, 48, 72, 96, and 120 h. Thereafter, groups of 4-5 rats were sacrificed and radiometric analyses were conducted on the following samples: blood, liver, kidneys, brain, spleen, skin, lungs, fatty tissue (from the abdominal cavity), urine, and stool. The samples were placed into polycarbonate ampules and incinerated in a current of oxygen at 700°C using an Oxymat IN-410 device (Intertechnique, France). The scintillator ZhS-8 was used in radiometric analysis of the samples.

Sample radioactivity was measured with an SL-4221 beta counter (Intertechnique, France), and expressed as cpm/g wet tissue. The resultant data were plotted in semilogarithmic coordinates. The half-time for the excretion of radioactive products ($t_{1/2}$) was calculated as follows: $t_{1/2} = 0.693/K_{el}$, where K_{el} is the elimination constant.

In series II experiments the drug was administered intragastrically for 35 days in a dose of 50 mg/kg (with adjustment for weight gain every ten days). On the 36th day the animals were treated with the labeled drug and subsequent protocol followed the single-dose scheme.

RESULTS

Figure 1 presents plots for changes in the specific radioactivity of the several organs, tissues, urine, and stools of rats after a single administration of ^3H-II. It is obvious that highest radioactivities were present in the liver and the lungs, and the lowest in the brain. One to three hours after administration of the drug the levels of radioactivity in the organs and tissues decreased, but the distribution pattern changed little.

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