ANTI-AMINE-OXIDASE ACTIVITY OF CERTAIN AMINE-PROPANOL DERIVATIVES[1]

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Two facts motivated our studies on the influence of 3-amine-2, 3-diphenyl-1-propanol threo- and erythro-isomeric forms on the mono-amine-oxidase activity. Firstly, the analysis of the pharmacological activity of these compounds revealed that besides their leio-myo-inhibitory action, as well as their antihistamine, antiacetycholine and antiserotonin effect on the smooth muscles of the intestines, they markedly stimulate the central nervous system of the experimental animals (5), accounting for a continuous increase of blood pressure. Moreover, a stronger nerve-stimulating action is exerted by the threo-isomeric form. Secondly, as illustrated in figure 1, the chemical structure of the amine-propanols studied allows their expression as analogues, though distant, of the phenyl-alkyl-amines.

If a comparison is made between their structure formula thus expressed and the formula of ephedrine (fig. 1), it becomes obvious that the OH and NH_2 groups, both participating as substitutes in the lateral alkylic chain of the ephedrine, are also present in the products investigated with the mere difference, that in the latter their places are interchanged. Furthermore, the lateral chain of the compounds subjected to pharmacological analysis is enriched with an additional phenyl radical attached to the second propanol carbon. As well known from literature reports (7, 9), some of the phenylalkylamines exert an inhibitory effect, in a stronger or weaker degree, on the monoamine-oxidase and account for excitation of the central nervous system.

Against the background of the latter consideration, we set ourselves the task to prove the effect of these compounds on the hepatic and cerebral mono-amine-oxidase (MAO), in experimental conditions, in vitro, by using the injection method for their introduction.

Experiments in vitro

We employed the mitochondria of rat's liver[2], obtained after the method of Schneider (8) and accordingly lyophilized after L. A. Bichin (1), as ferment source in our experiments in vitro. In compliance with the mono-

[1] We wish to express our gratefulness to B. Kurtev (and associates), corresponding member of the Bulgarian Academy of Science — Institute of Organic Chemistry for kindly placing at our disposal the threo- and erythro-isomers of 3-amine-2, 3-diphenyl-1-propanol.

[2] The hepatic mitochondria were received from the Laboratory for Biochemistry of amines and other nitrous substances of the Biological and Medical Chemistry Institute —

amine-oxidase activity recorded, the mitochondria obtained and duely lyophilized, were used in quantities ranging from 7,5 to 10 mg per sample, with substrate tyramine hydrochloride in terminal concentration 6 μ M per sample. We used 0,1 M potassium-sodium phosphate buffer, pH 7,4. The mono-amine-oxidase activity was determined by the quantity of amonia separated, measured in micormoles, in compliance with the method of its isotermic distillation and subsequent nesslerization (3, 6). Attempt was made to keep the concentration of the amine-propanols studied — 1×10^{-4} M, near to that produced in the animals during injection of the substance.

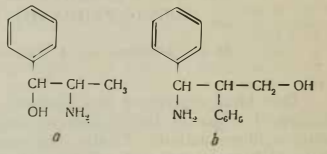

Fig. 1: *a*) ephedrine; *b*) 3-amine-2, 3-diphenyl-1-propanol

As a result of the experiments carried out, is was established that with tyramine hydrochloride as a substrate, the activity of hepatic mitochondrial mono-amine-oxidase is inhibited by the threo- and erythro-isomeric forms of the amine-propanol derivatives investigated. The threo-isomerate in concentration 1×10^{-4} M

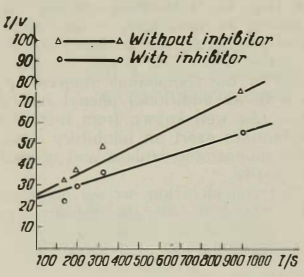

Fig. 2. Characteristic feature of threo-isomeric inhibition

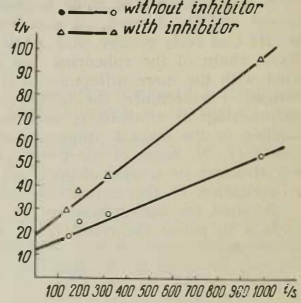

Fig. 3. Characteristic feature of erythro-isomeric inhibition

inhibits the ferment activity with an average of 27,2%, whereas the erythro-isomerate in the same concentration accounts for an inhibition of 22,5 per cent.

Academy of Medical Sciences — USSR, with chief V. Z. Gorkin, to whom we are willing to express our deep thanks and gratefulness.

Special experiments were set with the purpose of elucidating the mechanism of the hepatic mitochondrial mono-amine-oxidase inhibition, exerted by the two chemical compounds.

Without quantitative changes in the substances added, their inhibitory effect was tested with increased substrate concentrations (tyramine hydrochloride) from 1×10^{-3} to 1.4×10^{-2} M. A parallel series of experiments under analogous setting, but without inhibitor, estimated the ferment activity in conditions similar to the former.

The results of these experiments, elaborated according to the method of H. Lineover and D. Burk (4), and illustrated in figures 2 and 3, show that both isomeric forms of the amine-propanols investigated exert an inhibitory effect on the ferment, acting in a non-concurrent pattern.

Experiments in vivo

These experiments were carried out on a series of 48 male albino rats, weighing from 140 to 200 gr, according to a technique adapted to our conditions (9). The animals received no food for 16 to 24 hours prior to the experiment. On the day of experimentation intraperitoneal injection was performed of 50 mg/kg body weight threo- or erythro-isomers of 3-amine-2 3-diphenyl-1-propanol. Half an hour later the animals were sacrificed through decapitation. The brain was removed within 45—50 sec. and was collected in ice. In the following 45—50 seconds the liver was also removed and similarly collected in ice. All subsequent manipulations until incubation, were performed in previously cooled and kept in ice glass vessels (flasks).

Preparation of the homogenate:

a) from brain — the brains kept in the ice were freed from the covering membranes and blood vessels, and after drying on filter paper, they were weighed. After cutting in fine particles, they were quantitatively (in toto) transferred into the test tube of the homogenizer with teflon. 0,15 M borated-hydrochloric buffer was added, pH 8,50, containing 3% anion detergent OP-10 in a quantity securing the obtaining of 50% homogenate. The homogeneization lasted for approximately 1,5—2 minutes, at a rate about 2500 revolutions. The material obtained was passed through neutral filter consisting of a thin layer glass cotton UTV, under vacuum. The homogenate reaction was controlled, and in case of eventual 0,10—0,15 acidwise deviation, it was corrected by addition of 1 per cent water solution of potassium base;

b) from liver — first of all the liver was subjected to perfusion with previously cooled physiological solution until fully freed from the blood present therein. Next it underwent the treatment just described for the cerebral tissue.

The charging of the incubation vessels was carried out at a total volume 1,8 ml. Moreover, two vessels were charged for each homogenate — for control purpose, without substrate, containing 0,5 ml homogenate, 1,3 ml buffer (borated, 0,15 M, pH 8,50) and experimentally, with substrate, containing 0,5 ml homogenate, 0,2 ml substrate solution — tyramine hydrochloride in terminal concentration 6 μ M and 1,1 ml buffer.

Table 1

Formation of NH_3 in μM from Cerebral and Hepatic MAO with Tyramine Hydrochloride as Substrate in Terminal Concentration 6 μM per Sample

	Enzymatic source — Cerebral homogenate						Enzymatic source — hepatic homogenate					
	Formation of NH_3 in μM of Tyramine						Formation of NH_3 in μM of Tyramine					
	control animals		animals injected with ADFP		animals injected ADFP — erythro		control animals		animals injected ADFP — threo		animals injected ADFP — erythro	
	absolute quantity	change in %	absolute quantity	%	absolute quantity	%	absolute quantity	%	absolute quantity	%	absolute quantity	%
	2,10	—	2,76	+31,4	3,48	+65,7	6,80	—	4,92	−29,8	5,26	−24,90
	2,07	—	2,70	+30,4	3,62	+74,9	6,20	—	5,00	−35,9	5,20	−33,3
	2,39	—	2,63	+10,0	2,70	+13,0	4,46	—	4,60	+ 3,1	4,86	+ 9,0
	1,30	—	2,03	+56,2	2,59	+99,2	5,80	—	5,58	− 3,8	5,26	− 9,3
	2,37	—	3,70	+56,2	3,62	+52,7	6,06	—	4,26	−29,7	5,38	−11,2
	2,62	—	2,99	+14,2	3,23	+26,7	6,12	—	4,38	−28,4	5,48	−10,5
Average M ±m	2,14 ±0,17	—	2,97 ±0,21	+38,8	3,21 ±0,17	+50,0	5,91 ±0,24	—	4,69 ±0,18	−20,7	5,24 ±0,09	−11,3
Value of "t"			2,32		4,62				3,19		2,70	
Probability (p) with f=N−1			$p<0,10$ $p>0,05$		p=0,01				p=0,02		p=0,05	

With our setting therefore, 12 vessels were charged for each experiment: 2 vessels with brain homogenates for each of the 3 animal groups — control, treated with threo- and treated with erythro-isomer of the compounds investigated, and 2 for each of their hepatic homogenates.

The mixture was incubated in the Warburg apparatus for 45 minutes at 38 degrees C, 100 shakings per minute and gaseous environment — oxygen. The reaction of ammonia production in the vessels was fixed with trichloracetic acid in terminal concentration 5%, added as 50% solution — 0,20 ml, with a view to obtaining an ultimate volume of the sample 2 ml. The subsequent centrifugation for a duration of 5 — 10 min. at 10 000 revolutions/min yielded centrifugates with which the Conway cups were charged, two for each sample.

The charging of the Conway cups was carried out in the following manner: in the central space of each cup 1 ml 0,05 H sulphuric acid was placed, and in the main one — 0,6 ml centrifugate. After adding 2 ml saturated solution of potassium carbonate, the cups were hermetically sealed, without any delay. On the following day, after their uncovering, the content of the central space of each cup was washed with distilled water and quantitatively transferred into the test tube; 0,5 ml Nessler reactive was added, supplemented with distilled water up to exactly 10 ml. By means of photometry (FEK-M1-57) of the samples thus obtained and subsequent extinctions recording according to a previously set calibrated curve, the ammonia content therein was estimated in μ M.

Results

The data obtained underwent special elaboration according to variation statistics' rules and are presented through tabulation (Table 1). Their perusal at once draws the attention to the following fact: the mono-amine-oxidase of the liver is as much as three times more active than that of the brain — the ferment, in 0,5 ml hepatic homogenate having desaminated through oxidation almost completely the added substrate — 6 μM tyramine hydrochloride, has produced 5,91 μ M ammonia against 2,14 μ M ammonia, formed by the same quantity homogenate obtained from the brain.

Insofar the effect is concerned of the amine-propanol derivatives on the MAO, it is different, being determined by the organic origin of the ferment. Contrary to our expectations, they not only do not exert inhibitory effect on the cerebral MAO, but rather increase its activity in statistically reliable degrees as far as erythro-isomers are concerned. It is clearly evident that erythro-isomers enhance the activity of the ferment with 50% against 38,8% for the threo-forms. Besides that, the computed value of the test "t" is equal to 4,62 and with f=N—1 is present in the column of p = 0,01, which proves the inhibitory effect of the compound to be statistically reliable.

On the other hand, these substances inhibit the MAO activity in the liver, the threo-isomer appearing to be more active. The inhibitory effect resulting is statistically reliable with p=0,02, which is completely satisfactory. In this case "t" is equal to 3,19 and with f=N—1

is found in the column of p=0,02. Indeed, the effect of the erythro-amine-propanol derivative is weaker, but it too is statistically reliable, for the value of "t" is equal to 2,70 and with f=N—1 is present in the column of p=0,05.

Discussion

The results obtained are interesting from several viewpoints. First of all they raise the essential question for the difference between MAO in the liver and MAO in the brain, at least as regards their reaction to the various inhibitors. According to unpublished personal communications, this phenomenon is equally manifested towards such powerful inhibitors as iproniazid and tranylcipromin.

The results reported in this paper, both from the experiments with mitochondrial hepatic MAO in vitro, and from those in vivo, show that as far as strength is concerned of the inhibitory activity on the MAO in the liver of the two compounds investigated, it depends on the special configuration of their molecules. Therefore, it could be assumed on the basis of the experimental data obtained that the threo-disposition of the compound molecule, much more than the erythrodisposition, favours its interaction with the molecule of the ferment, which fact accounts for the reduction of its catalytic properties. It is necessary to point out however, that this peculiarity in the relations of the two isomeric forms of 3-amine-2, 3-diphenyl-1-propanol is observed in the hepatic MAO with tyramine substrate.

As far as the mechanism of inhibitory action on hepatic mono-amine-oxidase of the amine-propanols studied is concerned, the results obtained prove it to be independent from the threo-erythro-isomerics: in both cases the MAO inhibition occurs in a non-concurrent mechanism. That means that the ferment molecule acts reciprocally in a similar manner, both with the threo- and erythro-forms of the compounds investigated, with the only difference that its interaction with the threo-isomeric form is more effective insofar the degree of ferment inhibition is concerned.

On the other hand, the influence of these substances on the MAO activity in the brain is also worth of special attention. As already stated, it is activying in nature and furthermore, it is stronger with the erythro- as compared to the threo-isomeric form. The fact that the brain MAO is not inhibited under the effect of the amine-propanols studied by us, but on the contrary — it is activated, confirms the statement that not every stimulation of the central nervous system is connected with accumulation of mono-amines therein. In this respect, it will not be superfluous reminding that the stimulation which the substances in question provoke in the central nervous system, is different from that caused by the typical mono-amine-oxidase inhibitors in the following directions of the physiological parameters: it begins shortly after their introduction without a preliminary latent period, is accompanied by strongly increased reflex excitation of spinal-cord origin and is associated with tremor involving the entire body of the animal. It is not excluded that these peculiarities in the course of stimulation, caused by the threo- and erythro-forms of

the 3-amine-2, 3-diphenyl-1-amine-propanol, prove it not being conditioned by the accumulation of amines and more particularly catecholamines in the cerebral tissue.

Inferrences

The data reported in the paper show that threo- and erythro-isomeric forms of 3-amine-2, 3-diphenyl-1-propanol do have a determined influence on the MAO of hepatic and cerebral origin.

1) In experiments in vitro, these substances, in concentration 1×10^{-4} M, cause the inhibition of hepatic mitochondrial mono-amine-oxidase. A more strongly manifested inhibitory effect is observed with the threo-isomer.

2) The experiments in vivo show that the inhibition which these compounds exert on the activity of hepatic mitochondrial mono-amine-oxidase, occurs in a non-concurrent mechanism.

3) Their introduction in vivo equally brings about an inhibition of the hepatic mono-amine-oxidase activity. Here too, the stronger inhibitor appears to be the threo-form.

4) Their effect on the cerebral mono-amine-oxidase is activating. However, statistically reliable/relevant increase in the activity is recorded only with the erythro-isomeric form.

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ОБ АНТИАМИНООКСИДАЗНОЙ АКТИВНОСТИ НЕКОТОРЫХ АМИНОПРОПАНОЛОВЫХ ПРОИЗВОДНЫХ

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РЕЗЮМЕ

Авторы сообщают, что трео и еритро-изомерные форми 3-амино-2, 3-дифенил-1-пропанола как in vitro так и in vivo ингибируют активность печеночной моноаминооксидазы. Эффект трео изомера сильнее в обоих

случаях. Ингибирование фермента и в одном и в другом случае осуществляется по неконкурентному механизму.

На мозговую моноаминооксидазу трео форма не оказывает статистически существенного эффекта, а эритро повышает активность фермента.

Рассматривается значение трео-эритро-изомерии этого соединения о его влиянии на моноаминооксидазу печеночного и мозгового происхождения, как и отношение этих их эффектов к физиологическим феноменам, которые оны вызывают.