ON THE ATEBRIN INHIBITION OF THE OXIDATION OF ADRENALINE BY THE MONOAMINOOXIDASE

by

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The effect of adrenaline in the organism of homoiotherm (hot bodied) animals lasts, on account of his easy oxidability, but a short time. The colourless solution of adrenaline when exposed to the open air becomes yellow and finally brown due to the oxidation. The different tissues of the body posses enzymatic systems, which are capable to oxidize the adrenaline. The oxidation may occur at two places of its molecule: on the ring or on the chain with the production of ammonia.

The respiratory system of the cells, which contains catalytic iron i. e. the cytochrome-cytochromeoxidase system, is dehydrogenating adrenaline on the ring as was shown by Green and Richter[7]), and later by Keilin and Hartree[11]). According to these authors, by the removal of four hydrogen atoms, and by the following closing of the chain to form a ring, the first product of adrenaline oxidation — adrenochrome results. In the further stage of the oxidation the adrenochrome turns into a brown-pigmented substance. The above mentioned respiratory system is contained in every cell of the living tissue, and it is therefore clear that the adrenaline after having entered the body is beeing decomposed and its activity abolished. Green and Richter[7]) suppose that adrenaline is a substrate that is decomposed quickest by the cytochrome-cytochromeoxidase system. Ball, Cheen and Clark[2]) came to the conclusion that the adrenaline in the cells is oxidized first on the ring. Fahrländer[6]) after having observed very carefully the oxidation of the adrenaline by the cytochrome-cytochromeoxidase system, came to the following conclusion: a) The oxidation of adrenaline occurs as follows: adrenaline + fumaric acid → succinic acid + adrenochrome. It is clear that in this process the succinodehydrogenase plays a catalytic role. b) Green and Richter[7]) have shown another possible way for the oxidation of adrenaline: adrenaline → adrenochrome → cytochrome-cytochromeoxidase system → oxygen. Adrenochrome which is in this case acting as a hydrogen acceptor, would result, according to this supposition, from the equation at a). By combining these two ways into one simultaneous process a third process for the oxidative decomposition of adrenaline is obtained: c) adrenaline → succinic acid → succinodehydrogenase → adrenochrome → cytochrome — cytochromeoxidase system → oxygen. This processes are limited, acording to the author, only to the tissue of the liver of rabbits and rats, as the author does not mention the same processes going on in the whole animal.

The oxidative desamination of adrenaline was described the first time by Hare[10]) and after by Blaschko, Richter and Schlossmann[3]), who have noticed that by this oxidation ammonia is liberated. The oxidation results in the formation of aldehyde, ammonia and hydrogen superoxide. The monoaminooxidase which effects this oxidation oxidizes only the amines of the type $R\text{-}CH_2\text{-}NH_2$. The methylated amines are not affected. The methylation on the NH_2 group has no influence on the reaction. Hydroxyl group on the carbon atom diminishes the intensity of

the reaction. All compounds mentioned above do not belong to particular type of amines, and they therefore inhibit the monoaminooxidase effect by being adsorbed on the monoaminooxidase, which therefore becomes incapable to desaminate the above type of amines. The experiments with feeding and injecting of amines of the type $R\text{-}CH_2\text{-}NH_2$ conducted by Guggenheim and Löffler[8]), Richter[13]) and Ewins and Laidlaw[5]) especially with tyramine, showed results which demonstrate the effect of monoaminooxidase, for instance the corresponding carbonic acid has been excreted in the urine of the experimental animals. But concerning the adrenaline the results are not so clear. Weinstein and Manning[15]), as it is quoted, found in the urine of animals treated with adrenaline the protocatehic acid, while Richter[14]) having experimented on himself with oral application of adrenaline has found two third of the same compound bound on the sulphuric acid and perhaps on glucuronic acid in a hydrolysable form. The experiments on animals gave the same result and it is remarkable that adrenaline can pass through the intestine, liver and kidneys (all organs which contain most of the monoaminooxidase), without being changed.

Haas[9]), having worked in an isolated enzyme chain found that the oxidation was inhibited by atebrin which was substituted for riboflavine, resp. riboflavine-phosphoric acid as it disconnected the oxidative chain in carrying over the hydrogen from the substrate to the oxygen. One of us[1]) used this test to show that the oxidation of the succinic acid also, in the preparations of succinoxidase system, was inhibited by atebrin.

Monoaminooxidase is a enzyme, resistant to potasium cyanide, that occurs in many organs, but has a maximum concentration in the liver. Muscles do not contain this enzyme. Till today the isolation of the enzyme was not succesfull, nor could its qualities be closer analyzed. For being resistant to potassium cyanide, we supose that it belongs to the flavinezymes, and we shall try in this paper to prove it. For that reason we conducted experiments in hich we inhibited the enzyme with atebrin and tried to return its activity by adding riboflavine.

EXPERIMENTAL

Experimental animals were dogs, rabbits, guinea pigs and mice of both sex. Differences concerning sex were not observed.

Monoaminooxidase was tested on homogenized liver, heart, brain, kidney and muscle. These homogenates were diluted with a M/15 phosphate buffer pH = 7,4. The same tests were made with acetone powder from the organs mentioned. The later technique is to be preferred as it can be done with an utmost exactness and moreover the powder, even if kept at room temperature for weeks, maintains its activity.

The quantity of ammonia was determined according to Conway[4]). Tanret's solution composed of methyleneblue and methyl-red was used as an indicator in the titration as recomanded by Conway.

The blood pressure was measured with a mercury manometer, connected with the femoral artery by a glass cannula. In testing the activity of monoaminooxidase in certain organs in vivo, we used injections in the convenient arteries resp. veins. So for the liver in the portal vein, for the kidneys in the renal artery, for the brain in the carotide, for the heart in the abdominal hole vein and for the muscles in the femoral artery on the opposite side from the canula.

If during the determination of the production of ammonia by the oxidative desamination of adrenaline by the action of the monoaminooxidase of the liver tissue, we add to the Conway flask 1 ml of atebrin so that its final concentration becomes 10^{-3} M, the production of ammonia diminishes. We get

Table I

Each Conway-flask contains 1 ml of a mixture of homogenized tissue and M/15 phosphate buffer pH 7,4 and 1 ml of adrenaline (»Pliva«) 1 : 1000 (0,0055 M). After an incubation of 90 minutes at 40°, we add 1 ml of a saturated solution of potassium carbonate, and to the middle of the flask we give 1 ml of N/50 sulphuric acid. This system is again kept standing for 90 minutes on a temperature of 40°, till carbonate does not drive out the ammonia which binds itself on sulphuric acid. The data indicate the equivalents of oxygen calculated on the amount of ammonia by the supposition that to one molecule of ammonia correspond two atoms of oxygen or 15γ NH_3 = 10 mm^3 O_2. (The calculated amount of ammonia produced by the oxidative desamination of 1 mg adrenaline corresponds to 65.7 mm^3 of oxygen).

Guinea pig's	liver	kidney	brain	heart	muscle
The production of ammonia expressed in equivalents of oxygen in ml (10 mm^3O_2 = 15γ NH_3)					
without atebrin	68,20	56,11	18,24	10,80	2,20
with atebrin 10^{-3}M	11,33	6,25	2,21	0,0	0,0
inhibition in per cent	71,6	88,8	87,8	100	100
NH_3 in per cent of the calculated amount	>100	84,5	27,3	16,4	3,3
NH_3 in per cent of the measured amount	100	82,3	26,7	15,8	3,2

almost the same result when using acetone powder as when using homogenized tissues. The results of the experiments are shown on the Table I, where can be also seen that the monoaminooxidase is present in highest concentrations in the liver, kidney, then in the brain and heart, while it does not occur in the muscles of the skeleton. The results were the same whether the tissues of liver of the dog, rabbit, guinea pig or mouse was used. A typical result is given on the table mentioned.

The experiments made with the different organs of the dog, rabbit, guinea pig and mouse show as well as the results presented in Table I, that liver possesses the highest activity of monoaminooxidase. For this reason we used in further experiments only homogenized liver tissue resp. liver acetonic powder. The activity of the monoaminooxidase as it is shown at Table II does not differ essentially from the one when the liver from the animals mentioned was used.

Table II

Conditions as in Table I. The production of ammonia expressed in equivalents of oxygen

	Homogenized liver 1 ml aa with phosphate buffer pH 7,7				Liver acetonic powder 40 mg			
	dog	rabbit	guinea pig	mouse	dog	rabbit	guinea pig	mouse
	The production of ammonia expressed in equivalents of oxygen in mm^3 (10 mm^3 O_2 = 15γ NH_3)							
without atebrin	67.20	68.13	66.16	66.96	65.81	66.22	66.26	65.66
with atebrin	12.13	12.20	14.82	11.82	10.50	10.16	11.21	10.62
Inhibition in per cent	81.9	82.1	83.3	82.3	84.0	84.7	83.1	83.8

Atebrin as it is seen on Table I and II is able to inhibit the activity of monoaminooxidase by ca 70 to 100 per cent when present in 1×10^{-3} M of final concentration. According to Haas[9]) this inhibition is nothing else but a competition with riboflavine. To prove this statement we tried to restore the activity of the enzyme with riboflavine (chemically pure »Ro-

che«). As the results in Table III show, the restoration of the activity of the enzyme, viz. the prevention from the atebrinic inhibition almost always succeds by 100 per cent. From this results we conclude, by analogy to H a a s, that the monoaminooxidase is probably a flavin-enzyme.

Monoaminooxidase was described former as a cyanide resistant enzyme, and our experiments show that it can be

Table III

Conditions as in the Table I. The experiments are with 40 mg of liver acetonic powder. The numbers indicate the production of ammonia in equivalents of oxygen in mm^3

	dog	rabbit	guinea pig	mouse
without atebrin	66.86	68.20	66.16	66.58
with atebrin	12.81	13.10	13.62	11.88
with atebrin and riboflavine	66.82	68.24	66.12	66.46
with riboflavine	68.86	66.19	66.17	66.52
inhibition in per cent	80.8	80.8	79.4	82.2
restitution of activity in per cent	99.9	100	99.9	99.7

classified as a flavin-enzyme. Isolation of the enzyme and its further investigation will throw more light on its detailed structure, particularly its prosthetic group.

Oxidations as tested in vitro do not show us always authentical results. The circumstances and conditions in vivo are quite different, the influence of many other substances is important, and to imitate and reproduce the whole system in vitro is still impossible today.

For this reason we were interested to find out if it would be possible to obtain the results mentioned above also in vivo. To investigate this we used the effect of adrenaline on the blood pressure. As it is known the blood pressure suddenly rises, following the intravenous application of the drug, keeps standing on a certain level for some time and then drops. The blood pressure drops because the adrenaline is being oxidized on the ring and desaminated on the chain.

We have suposed that if atebrin is inhibiting the effect of the monoaminooxidase, than the effect of adrenaline on the blood pressure (the time of rised pressure) would last longer and perhaps riboflavine could partly renew the activity of the monoaminooxidase by shortening again the time during which the blood pressure is rised.

In the attempt to get an answer to this question we applied atebrin and riboflavine in the same way as adrenaline in our investigations by injecting them: for the liver in the portal vein, for the kidney in the renal artery, for the brain in the carotide comm., for the heart in the abdominal hole vein and for the muscles in the femoral artery. The blood pressure was in every case registrated through a cannula in the femoral artery at the opposite side. The results of the experiments give us a hint about the differences in the duration of the time of rised blood pressure, when adrenaline was injected in different organs. We have seen that the shortest effect on the blood pressure occured in the case, when adrenaline was applied on the liver through the portal vein. This effect is nearly equal to the one obtained when adrenaline was applied on the kidneys by injecting it into the renal artery. On the muscle it lasts a little longer, and by the application to the other organs it lasts much longer, which indicates a lower activity of the monoaminooxidase and the other enzymes which oxidize adrenaline. When we repeated the experiment by injecting adrenaline after atebrin, the result was entirely different. The application of adrenaline into the kidneys and liver show the largest prolongation of the increased blood pressure, on the muscle and the heart there was no prolongation while in case of the brain the prolongation was minimal or absent entirely.

If we suppose that atebrin competes with the prosthetic group of the flavin enzyme, we can conclude that this prolongation occurs probably in the following way: liver and kidneys are the organs in which — as it seems — adrenaline is being oxidized most rapidly. The oxidation occurs either on the ring through the oxidative systems pointed out by F a h r l ä n d e r[6]) or at the chain through monoaminooxidase. As the liver and kidneys are richest in both, the rised blood pressure persists for the shortest time after the injection of adrenaline into the portal vein and the renal artery. Meanwhile it was proved that the oxidation in the liver and the kidneys is effected to a higher measure by the way of desamination at the chain than is the case in other organs. Therefore a prolongation of the action of adrenaline on the blood pressure is maximal by administrating the drug in the liver and kidney just after atebrin.

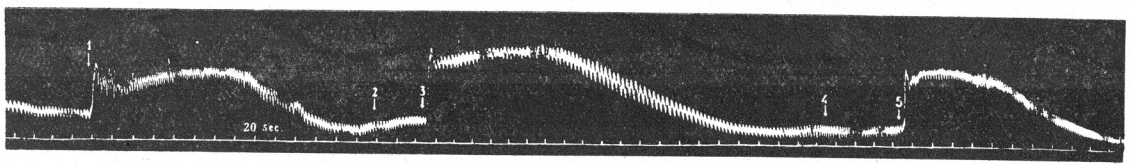

Fig. 1. The registration of blood pressure on a dog of 12 kg. bodyweight. Cannula in the femoral artery of the left side. At arrow 1 injection of 1 mg adrenaline into the portal vein, at 2 injection of 0.3 g atebrin, at 3 1 mg adrenaline, at 4 0.3 g riboflavine and at 5 the mentioned amount of adrenaline. Adrenaline given after atebrin acts longer, riboflavine shortens again its action. (See text)

Tabela IV

Conditions as in Table I, II and III.

The amount of ammonia produced by the oxidative desamination of adrenaline by liver, kidney, heart, muscle and brain of rabbit and guinea pig treated with atebrin, atebrin + riboflavine and riboflavine as stated. To each flask was added 1 ml of adrenaline solution containing 1 mg of adrenaline »Pliva«

	rabbit's					guinea pig's				
	liver	kidney	heart	muscle	brain	liver	kidney	heart	muscle	brain
	the production of ammonia expressed in equivalents of oxygen in ml (10 mm^3 O_2 = 15γ NH_3)									
untreated	66.61	55.21	4.26	1.16	14.1	65.54	54.11	6.61	2.21	11.88
treated with atebrin	12.18	7.42	0.0	0.0	2.18	13.14	4.48	0.0	0.0	2.81
treated with atebrin and riboflavine	66.59	55.24	4.81	1.12	13.92	65.43	53.82	6.64	1.92	11.00
treated with riboflavine	66.64	54.90	4.88	0,92	15.1	65.83	54.22	6.02	2.62	12.14

The fourth group received only the corresponding amount of physiological salt solution. One day after the last injection the animals were sacrificed by bleednig and the aceton powders resp. homogenates of the organ tissues were prepared. The rate of oxidative desamination was determined and, as it is shown on table IV, atebrin administrated in vivo inhibited the desamination of adrenaline in vitro. The results with regard to the inhibition and the activity of monoaminooxidase in different organs agree with those presented on Table I, II and III.

CONCLUSIONS

Experiments with the oxidative desamination of adrenaline using homogenized tissues resp. acetonic powders of the tissues of different organs were carried out to demonstrate the qualities of the monoaminooxidase, which performs this desamination. As mentioned above, it was known that its activity remains unimpaired after the addition of potassium cyanide. Due to this, it was presented as a cyanide resistant oxidase. H a a s' test with which it was possible to point out the competition between atebrin and flavine as the prosthetic group of the flavine enzyme, could be applied, as can be seen from the experiments above, to prove the activity of monoaminooxidase. This oxidase is not present in every tissue, nor is it present in the same concentration in all tissues which possess the ability to destroy adrenaline by liberating ammonia.

Adrenaline can be oxidized in two ways, oxidation on the ring produces adrenochrome and the oxidative desamination gives aldehyde. The structure of the final oxidized product is unknown. It is also unknown whether the intermediary product resp. the final product itself has any physiological importance. According to hypothesis of G r e e n and R i c h t e r it seems that adrenochrome acts as a hydrogen acceptor in vital oxidations, and in such a way participates also in the oxidation of the adrenaline itselt. This oxidation of the adrenaline to adrenochrome can be effectuated by many oxidative enzymes. We may ask the question whether the products resulting from the action of monoaminooxidase on the adrenaline possess also a physiological importance. The answer to this as well as to the other question of possible other functions of adrenochrome is a matter of further investigations and is worth while a careful exploring.

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IZVOD

O atebrinskoj inhibiciji oksidacije adrenalina s pomoću monoaminooksidaze

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Pokusima po Conway-u sa acetonskim praščima i homogenatima tkiva organa psa, kunića, zamorca i miša pokazali smo da atebrin sprečava oksidativnu dezaminaciju adrenalina. Ova dezaminacija najjače je izražena kod homogenata i acetonskih prašaka jetre i bubrega, dok je kod tkiva mozga, srca i naročito mišića neznatna. Dezaminaciju provodi monoaminooksidaza i tamo, gdje je ima najviše, atebrin i najjače sprečava njezinu aktivnost. Dodatkom kemijski čistog riboflavina ponovno se obnavlja aktivnost ovog fermenta. Iz ovog se zaključuje, da je monoaminooksidaza flavin-ferment. Gornje smo rezultate potvrdili pokusima in vivo uz registraciju krvnog tlaka i uštrcavanje adrenalina, atebrina i riboflavina u pripadajuće dovodne krvne žile istraživanih organa. Atebrin uštrcan prije adrenalina produljuje njegov učinak na krvni tlak, a naknadno ili prethodno uštrcavanje riboflavina uspostavlja ponovno normalni adrenalinski učinak. Acetonski prašci ili homogenati tkiva životinja, koje su za života bile zasićene atebrinom izgubile su dijelom aktivnost monoaminooksidaze, a ako su uz atebrin dobivale i riboflavin aktivnost fermenta u njihovim tkivima ostala je sačuvana u punoj mjeri.

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