Brit. J. Pharmacol. (1952), 7, 99.

AMINE OXIDASE IN THE IRIS AND NICTITATING MEMBRANE OF THE CAT AND THE RABBIT

BY

JUDITH ROBINSON

From the Department of Pharmacology, Oxford University

(Received October 11, 1951)

It has been known for some time that the normal nictitating membrane of the cat's eye is much less sensitive to noradrenaline than to adrenaline. Bacq (1937) explained this difference by supposing that the methylation of noradrenaline not only conferred inhibitory properties on noradrenaline but enhanced its excitatory action. On the denervated nictitating membrane there is no such difference, and both adrenaline and noradrenaline produce a large contraction nearly always greater than that produced by adrenaline in the normal membrane. Thus the ratio of the contraction in the denervated membrane to that in the normal membrane was found by Bülbring and Burn (1949) to be much greater for noradrenaline than for adrenaline.

A similar situation exists in the pupil of the cat's eye. Greer, Pinkston, Baxter, and Brannon (1938) observed that noradrenaline had much less dilator effect than adrenaline in the normal eye. Burn and Hutcheon (1949) showed that after denervation the sensitivity to noradrenaline was increased much more than that to adrenaline, so that noradrenaline was almost as effective in dilating the denervated pupil as adrenaline. They suggested that the phenomena in the nictitating membrane and in the pupil could be explained if there was an enzyme in these structures specially adapted to destroy noradrenaline. This might well be true since noradrenaline is the sympathetic transmitter. Burn and Hutcheon suggested that injected noradrenaline was ineffective in the normally innervated structure because it was rapidly destroyed, and that adrenaline was more effective because it was less rapidly destroyed. In the denervated tissue they supposed that the enzyme diminished in amount, and thus both substances produced a greater effect.

Various steps were required to prove the truth of this hypothesis. The first was to demonstrate the presence of an enzyme and to identify it. The second was to show that the enzyme has a greater affinity for noradrenaline than for adrenaline.

Evidence has already been published relating to the second point; Burn and Robinson (1951) showed that amine oxidase has a greater affinity for noradrenaline than for adrenaline. Therefore it was important to discover whether this enzyme was actually present in the nictitating membrane and the iris. This paper describes the investigation of this question.

Experiments Performed

Nictitating membranes.—A test for amine oxidase has been described by Florence and Schapira (1945) in which the presence of the enzyme is detected by its

conversion of phenylethylamine into the corresponding aldehyde, phenylacetaldehyde, which is recognized by its characteristic smell of hyacinths.

The nictitating membranes were removed from a spinal cat by cutting round the orbit with a scalpel and dissecting them off the eyeball. A suspension was made by cutting the tissue with scissors and pounding in an ice-cold mortar alternately, and adding 4 ml. 0.067 M-sodium monohydrogen phosphate buffer of *p*H 7.4 for each membrane. To 2 ml. of this suspension 0.2 ml. 0.01 M-phenylethylamine hydrochloride was added, while a control sample had 0.2 ml. water. The tubes were closed with rubber stoppers, incubated at 37.5° C., and left for an hour.

When the tubes were opened, the sample incubated with the amine had a faint but characteristic smell of hyacinths, showing that there had been oxidation of the amine to aldehyde.

Manometric observations.—Quantitative estimations were made manometrically by Warburg's method on the nictitating membranes of both cats and rabbits. Rabbits were killed by a blow on the neck and bleeding out. Cats were either gassed with coal gas or anaesthetized with sodium amytal and then bled out. The tissues were removed immediately and stored at −15° C. until the estimations were performed. For the manometric experiments conical manometer flasks with one side bulb and a potash tube were used. In each experiment an " enzyme blank " and one or more samples with added substrates were set up. In most experiments tyramine served as substrate, as it is the amine most readily oxidized by amine oxidase ; 1.6 ml. of the tissue suspension and 0.2 ml. 0.1 M-KCN, the latter being neutralized to bromthymol blue with N-HCl, were put into the main compartment ; the potash tube contained 0.3 ml. of the 2 N-KCN-KOH mixture described by Umbreit (1945).

The side-bulbs contained either 0.2 ml. water or 0.2 ml. 0.1 M-tyramine hydrochloride. The cyanide was added in order to exclude oxidation reactions other than that due to the amine oxidase system, which is not inhibited by cyanide. The flasks were filled with O_2 and incubated at 37.5° C. Under these conditions the blank oxygen consumption of the enzyme without substrate was always small, but in the presence of tyramine there was always a marked increase of oxygen uptake. Readings were taken at 5-min. intervals during the first 30 min. and plotted against the time ; the enzyme activities were calculated from the shape of the graph during that period. Results were expressed in μl. O_2 consumed per hour by one membrane. This method was adopted as it was somewhat difficult to exclude adhering connective tissue, and it was thought better to determine total activity rather than activity per unit weight.

The results obtained in the experiments with nictitating membrane from cats are given in Table I. The figures obtained in four experiments with rabbit nictitating membrane were of the same order.

In the experiments on iris the tissue preparation and manometric procedure were similar, except that six irises were used in each experiment and were suspended in 3.5 ml. of the phosphate buffer. In these experiments the oxygen uptake without added substrate was larger, but the additional oxygen uptake with substrate was always at least double. The values are summarized in Table II. The enzyme activities were again calculated from the readings for the first 30 min. and expressed as μl. O_2 per g. tissue per hr.

Similar figures were obtained in five experiments with a rabbit iris suspension.

Since this tissue contains melanophores it was desirable to exclude the possibility that a polyphenol oxidase type of enzyme contributed to the oxidation of

TABLE I

OXIDATION OF TYRAMINE BY SUSPENSION OF CAT NICTITATING MEMBRANE

Exp. No.	μl. O_2 consumed per hr. per membrane		Exp. No.	μl. O_2 consumed per hr. per membrane	
	Without substrate	Additional consumption with tyramine		Without substrate	Additional consumption with tyramine
1	11	70	8a	9.5	38
2	17	66	8b	20	57
3	0	61	9a	0	52
4	15	100	9b	14.5	53
5a	5	79	10a	20	97
5b	6	78	10b	24.5	129
6a	0	49	11a	19	61
6b	1.5	59	11b	18	81
7a	12.5	105	12a	24.5	79
7b	14	99	12b	27	98

TABLE II

OXIDATION OF TYRAMINE BY SUSPENSIONS OF CAT IRIS

Exp. No.	μl. O_2 consumed per g. per hr.	
	Without substrate	Additional consumption with tyramine
1	39.5	123
2	50	192
3	29.5	118
4	53	124
5a	33.5	162
5b	52	165
6a	40	205
6b	55	195

tyramine. In one experiment, therefore, *iso*amylamine hydrochloride was used as substrate. This amine was also oxidized, the activities being :

without substrate ... ... 43 μl./g./hr.
additional uptake with tyramine ... ... 312 μl./g./hr.
additional uptake with *iso*amylamine ... ... 154 μl./g./hr.

The relative activity of the amine oxidase in liver towards tyramine and *iso*amylamine is similar to that found in this experiment.

It was of particular interest to find whether adrenaline and noradrenaline were oxidized. In some preliminary experiments it was found that both amines were oxidized but at a much slower rate, and it was for this reason that tyramine was adopted as the chief substrate. In the presence of 0.01 M-KCN and 1.6 mg. cysteine per flask autoxidation was inhibited (Blaschko, Richter, and Schlossmann, 1937). However, the addition of cysteine increased the oxygen consumption of the enzyme blank to such an extent that the determinations had to be given up as unsatisfactory.

It is interesting to note that in liver preparations the addition of cysteine had no similar effect on the blank oxygen consumption. Cyanide alone appeared to be sufficient to prevent autoxidation of adrenaline and noradrenaline for the first 30 min. of incubation.

Pentamidine is known to be a strong inhibitor of amine oxidase (Blaschko and Duthie, 1945). In one experiment on rabbit iris with tyramine as substrate 0.2 ml. of 0.01 M-pentamidine isethionate was added to the main compartment. In this experiment the oxygen consumption in the absence of pentamidine was 35.5 μl. in the first 30 min. and in the presence of pentamidine it was 2 μl. Thus the inhibition in 0.001 M-pentamidine was over 90 per cent.

Discussion and Summary

The experiments described above show that amine oxidase is present in the iris and nictitating membrane of cats and rabbits. The enzyme oxidizes the amines tyramine, adrenaline, noradrenaline, and *iso*amylamine ; the activity remains in the presence of cyanide ; and it is inhibited by pentamidine. These properties are characteristic of the enzyme amine oxidase.

This evidence adds to that recently obtained by Thompson and Tickner (1951) of the presence of amine oxidase in the large and small arteries and veins of the rabbit. Amine oxidase is evidently an enzyme of greater importance than Bacq concluded when he wrote in 1949 on the basis of the evidence available at that time.

The presence of amine oxidase in the nictitating membrane makes it probable that the sensitization of that organ to adrenaline by cocaine, and the much greater sensitization to noradrenaline, are to be explained as due to the inhibition of amine oxidase by cocaine (Philpot, 1940).

I should like to thank Professor Burn and Dr. Blaschko for their help and advice during this work.

References

Bacq, Z. M. (1937). *Arch. int. pharmacodyn.*, **55**, 190.
Bacq, Z. M. (1949). *Pharmacol. Rev.*, **1**, 1.
Blaschko, H., and Duthie, R. (1945). *Biochem. J.*, **39**, 347.
Blaschko, H., Richter, D., and Schlossmann, H. (1937). *J. Physiol.*, **90**, 1.
Bülbring, E., and Burn, J. H. (1949). *Brit. J. Pharmacol.*, **4**, 202.
Burn, J. H., and Hutcheon, D. E. (1949). *Brit. J. Pharmacol.*, **4**, 373.
Burn, J. H., and Robinson, J. (1951). *Brit. J. Pharmacol.*, **6**, 101.
Florence, G., and Schapira, G. (1945). *C.R. Soc. Biol., Paris*, **139**, 36.
Greer, C. M., Pinkston, J. D., Baxter, J. H., jun., and Brannon, E. S. (1938). *J. Pharmacol.*, **62**, 189.
Philpot, F. J. (1940). *J. Physiol.*, **97**, 301.
Thompson, R. H. S., and Tickner, A. (1951). *J. Physiol.*, **115**, 34.
Umbreit, W. W. (1945). *Manometric Techniques and Related Methods for the Study of Tissue Metabolism*, p. 47. Minneapolis : Burgess Publishing Co.