J. Physiol. (1951) 115, 34–40

THE OCCURRENCE AND DISTRIBUTION OF MONO-AMINE OXIDASE IN BLOOD VESSELS

By R. H. S. THOMPSON and A. TICKNER

From the Department of Chemical Pathology, Guy's Hospital Medical School, London

(*Received* 24 *February* 1951)

It has been known for some time that the body possesses a number of different enzymic mechanisms which might be responsible for the inactivation of adrenaline and other amine derivatives of catechol inside the body. Such compounds can be converted into derivatives showing little or no pressor activity by (1) oxidation of their phenolic hydroxyl groups, (2) oxidative deamination of the side-chain by a mono-amine oxidase, or (3) esterification.

A consideration of the pharmacological properties of ephedrine, together with the finding by Blaschko, Richter & Schlossmann (1937*a*) that ephedrine inhibits the destruction of adrenaline by mono-amine oxidase, although it is not itself oxidized by it, led Gaddum & Kwiatkowski in 1938 to put forward the view that many of the actions of ephedrine can be explained on the assumption that it is playing a role with respect to adrenaline and mono-amine oxidase analogous to that played by eserine in respect to acetylcholine and cholinesterase. Such a view would imply that mono-amine oxidase must be concerned with the destruction of adrenaline under physiological conditions, and that this enzyme may be distributed in relation to adrenergic nerve endings in much the same way as cholinesterase may be assumed to be related to cholinergic nerve endings.

Part of Gaddum & Kwiatkowski's evidence for this hypothesis was obtained from studies on the perfused vessels of the rabbit's ear, and their interpretation of these experiments was given added force by the earlier observations of Schaumann (1928) and Burn (1932) that ephedrine exerts hardly any vasoconstrictor action on perfused tissues in the absence of adrenaline. If this interpretation of the mode of action of ephedrine is correct, it should therefore be possible to demonstrate the presence of mono-amine oxidase in the blood vessels of the rabbit's ear and elsewhere. Bhagvat, Blaschko & Richter (1939) were unable to find a mono-amine oxidase in the skin of rabbit's ears, and Richter & Tingey (1939) attempted unsuccessfully to demonstrate activity in

a sand-ground preparation of rabbit ears which had been freed from hair and cartilage. In the materials used by each of these groups of workers, however, blood vessels and adrenergic nerve endings will clearly constitute only a small part of the total tissue, and no experiments appear to have been done by them on blood vessels freed from other tissue.

Using a qualitative test (Florence & Schapira, 1945) for the presence of mono-amine oxidase in tissue extracts based on identifying the odour of hyacinths due to phenylacetaldehyde produced by this enzyme when acting on phenylethylamine, Schapira (1945), however, succeeded in showing mono-amine oxidase activity in the rabbit's ear and also in the jugular vein of the dog. He does not appear to have carried out this test on isolated arteries.

We had recently demonstrated the presence of a highly active mono-amine oxidase in placenta (Thompson & Tickner, 1949); in view of the vascular nature of this tissue we became interested, as a result of conversations with Dr R. T. Grant, in the possible association of this enzyme with vascular structures generally, and decided therefore to study isolated blood vessels of different kinds, including the vessels of the rabbit's ear, using both manometric and titrimetric methods for the identification and assay of the enzyme.

EXPERIMENTAL

Material

For ease of dissection, and in order to obtain adequate amounts of fresh material for assay, most of the present work was done on rabbits. Immediately after killing the animals (either by stunning or by air embolism), the vessels to be examined were dissected out and placed in 0·9% NaCl. Adherent connective tissue which had not been stripped from the vessels while *in situ* was then removed by further dissection of the vessels pinned out on cellophane over a cork mat; any contained blood was squeezed out of the cleaned vessels while lying in saline. The tissue was then finely minced with scissors.

The materials used in the various experiments comprised: the whole length of the aorta and common carotid, pulmonary and renal arteries, the brachial artery from the subclavian to near the elbow joint, the femoral artery from the iliac vessels to near the ankle, and the central artery of the ear; when veins were used, the superior vena cava, the inferior vena cava, the jugular and subclavian vessels were dissected and pooled.

In some of the experiments with rabbit aorta the cleaned vessel was slit open longitudinally and pinned down on cellophane; the inner half to two-thirds of the vessel was then scraped off the remaining outer part, which was discarded. This was done in order to limit the material under investigation to the tunica interna and tunica media of the artery, so that we should not include in our assay any enzyme which might be present in association with nerve plexuses or other structures in the adventitia or surrounding it.

A few experiments have also been carried out on guinea-pig and rat aortas, and also on the small muscular branches of the popliteal artery in a human limb obtained immediately after amputation on account of gangrene of the foot.

Estimation of enzymic activity

Oxygen consumption was determined manometrically using the Warburg apparatus. Between 30 and 100 mg. of the minced blood vessel was used in each Warburg flask.

Tyramine hydrochloride (L. Light and Co. Ltd.) was used as substrate in 0·01 M. solution except where otherwise stated.

Measurements were made at 38° C. in 0·067 M-phosphate buffer (pH 7·3), and in a gas phase of O_2, over a period of 2 hr., the results being expressed as μl. O_2/g. tissue (wet wt.)/hr. Controls for non-enzymic oxidation of the substrate, and for the O_2 consumption of the enzyme preparation in the absence of added substrate, were included in each experiment.

Ammonia production in the course of the reaction was measured in some experiments. At the completion of measurement of O_2 uptake, 1 ml. of 25% trichloroacetic acid was added to each Warburg flask, and the contents decanted into a 15 ml. centrifuge tube. Each flask was washed once with 1 ml. of 5% trichloroacetic acid which was then added to the centrifuge tube. Ammonia was estimated in 1 ml. of the centrifuged supernatant fluid by steam distillation using the Markham (1942) apparatus.

RESULTS

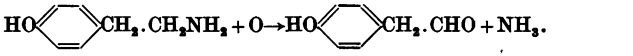
The extra oxygen consumption of the different preparations in the presence of 0·01 M-tyramine hydrochloride is shown in Fig. 1. Owing to the relatively small size of some of the vessels studied, it was necessary in most of the experiments to pool the material from two or more rabbits; even so, it was felt advisable in view of the low weights of, for example, ear or renal arteries to extend our measurement of oxygen consumption over a 2 hr. period. A slight fall in the rate of oxygen uptake was noticed during the second hour.

Fig. 1 shows that for any given vessel fair agreement was obtained in the level of enzymic activity in the different experiments. On the other hand, the values and their means, obtained for the different vessels, differ noticeably. It would seem that the vessels which we have studied may be divided into two groups, the aorta and the carotid, renal and pulmonary arteries having a considerably higher activity than the brachial, femoral and ear arteries and the great veins (superior and inferior vena cava and jugular and subclavian veins).

On account of the difficulty of ensuring that the vessels were dissected completely free from all surrounding tissue, we thought it important to discover at an early stage in the work whether the enzymic activity which we were demonstrating was situated in the vessel wall itself or whether it was due to the inclusion in our material of nerve plexuses or other structures in the adventitia or surrounding tissue which we had not completely removed. As described earlier, in two experiments we therefore dissected off the inner half to two-thirds of a portion of the aorta, and compared this inner part of the vessel with other samples of the whole thickness of the aorta. It will be seen that in these two experiments the inner portion gave values which were slightly higher than those obtained with the whole thickness of the aorta, indicating that our observations are not due to the inclusion of extra-arterial tissue.

A few experiments have also been done with rat and guinea-pig aorta; both these tissues were found to be active, showing extra O_2 uptakes in the presence of tyramine of 408 and 401 μl. O_2/g./hr. respectively. In one experiment, carried out on small muscular branches of the popliteal artery dissected from a freshly amputated human leg, a value of 303 μl. O_2/g./hr. was obtained.

If the observed oxygen uptake in the presence of tyramine is due to an amine-oxidase in the arterial wall, ammonia and *p*-hydroxyphenylacetaldehyde will be produced in accordance with the following overall equation:



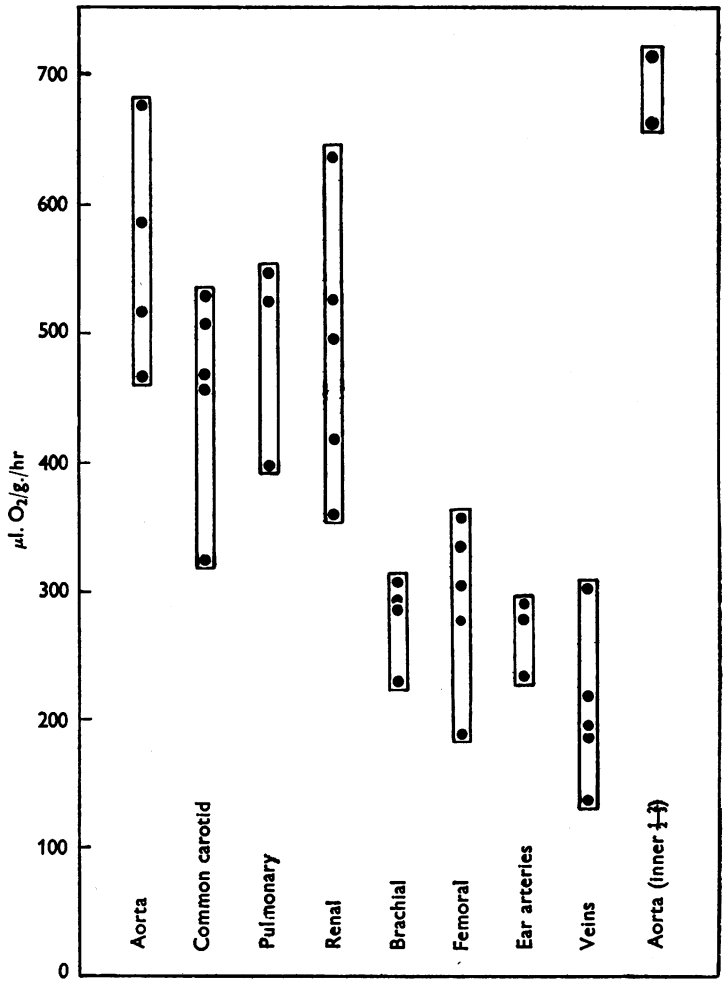


Fig. 1. Mono-amine oxidase activity of rabbit blood vessels (0·01 M-tyramine hydrochloride as substrate).

In a series of experiments we have therefore carried out ammonia estimations on the protein-free filtrate of the contents of the Warburg flasks at the end of the measurement of oxygen consumption.

Since it is to be expected that the aldehyde which is formed during the oxidation of the tyramine by a mono-amine oxidase may undergo further oxidation, we have also estimated the oxygen uptake and ammonia formation in flasks to

which 0·001 M-KCN had been added to prevent further oxidation; KCN was also added to the centre cup of the Warburg flasks to prevent loss of cyanide from the main chamber. The results of these experiments are shown in Table 1.

In agreement with the above equation it will be seen that in the presence of cyanide to prevent further oxidation of the aldehyde approximately 1 mole of ammonia is produced for each atom of oxygen utilized.

As further confirmatory evidence that we are dealing with an oxidative deamination of the tyramine by a mono-amine oxidase, we tested for the

TABLE 1. Ratio of atoms oxygen utilized/mol. ammonia produced and ratio utilized in the presence and absence of cyanide during oxidation of tyramine by rabbit aorta

(O_2 and NH_3 expressed as μmol./g./2 hr.)

	With cyanide			Without cyanide			
Exp.	μmol. O_2 (*a*)	μmol. NH_3	Atoms O / mol. NH_3	μmol. O_2 (*b*)	μmol. NH_3	Atoms O / mol. NH_3	Ratio of O_2 uptakes (*b*/*a*)
1	—	—	—	41·6	47·7	1·75	—
2	17·0	33·6	1·01	28·1	39·2	1·44	1·65 : 1
3	34·0	61·2	1·11	57·2	73·0	1·56	1·68 : 1
4	17·6	31·9	1·13	29·3	43·2	1·36	1·67 : 1
5	23·1	46·6	0·99	—	—	—	—

TABLE 2. Inhibition of oxidation of tyramine (0·01 M.) by rabbit aorta mono-amine oxidase

Inhibitor	Conc. inhibitor (M.)	Inhibition (%)
Ephedrine hydrochloride	0·02	61
Benzedrine sulphate	0·0025	71
Benzedrine sulphate	0·005	75
Benzedrine sulphate	0·015	83
Procaine hydrochloride	0·0175	42
Nupercaine hydrochloride	0·0025	57
Nupercaine hydrochloride	0·005	80
Methylene blue	0·01	84
sec-Octyl alcohol	0·005*	49

* Added as a suspension in phosphate buffer to give a final concentration of 0·005 M., assuming complete solution.

presence of the aldehyde in the contents of the Warburg flasks at the end of the period of incubation at 37° by means of the 2 : 4-dinitrophenylhydrazine alkali test as described by Pugh & Quastel (1937); a positive result was obtained in those flasks containing both enzyme and tyramine.

In view of Gaddum & Kwiatkowski's original observations (1938) on ephedrine, it was obviously of interest to study the action of ephedrine and of other recognized inhibitors of mono-amine oxidase on the enzyme in blood-vessel walls. In addition to ephedrine, we have studied the effect of benzedrine, which was shown by Blaschko (1940) to be a more powerful inhibitor than ephedrine, procaine and nupercaine (Philpot, 1939), methylene blue (Philpot, 1937; Philpot & Cantoni, 1941) and *sec*-octyl alcohol (Blaschko *et al.* 1937*a*). The percentage inhibitions of the mono-amine oxidase of rabbit aorta caused by these various compounds are shown in Table 2.

In confirmation of Blaschko's finding (1940), benzedrine is also a more potent inhibitor than ephedrine when tested on the enzyme in rabbit aorta; since benzedrine sulphate was the compound used in these experiments, equivalent concentrations of sodium sulphate were added to the control flasks to correct for any possible effect of the sulphate ion. The other compounds studied all showed themselves to be powerful inhibitors in the concentrations used.

In most of our experiments tyramine hydrochloride was used as substrate, since this compound does not undergo any appreciable non-enzymic oxidation under the conditions used. It was thought desirable to demonstrate that this enzyme in blood vessels is also active against the physiological substrates, adrenaline and noradrenaline. When using these compounds, 0·001 M-potassium cyanide was present in the flasks to prevent oxidation by the cytochrome oxidase system; 0·0043 M-reduced glutathione was also added to minimize autoxidation (Blaschko, Richter & Schlossmann, 1937*b*).

The results obtained in these experiments are shown in Table 3.

TABLE 3. Extra O_2 uptake of minced rabbit aorta in presence of adrenaline, noradrenaline and tyramine together with KCN (0·001 M.)

(Substrate conc. 0·01 M.; results expressed as μl. O_2/g./hr.)

	L-Adrenaline hydrochloride (B.D.H.)	L-Noradrenaline bitartrate (Bayer Products Ltd.)	Tyramine hydrochloride
	141	74	344
	72	44	258
	84	105	394
Mean	99	74	332

In the presence of cyanide the rate of oxidation of tyramine amounts to only about 60% of the rate in its absence (see Table 1). Adrenaline is oxidized, under these conditions, at about one-third the rate of tyramine; L-noradrenaline is also oxidized, at about the same rate, or perhaps rather more slowly than adrenaline, a finding which agrees with the earlier observations of Blaschko *et al.* (1937*a*) on the rate of oxidation of DL-noradrenaline by guinea-pig liver and intestine.

DISCUSSION

Despite the earlier statements that mono-amine oxidase activity is not detectable in the rabbit's ear the experiments described above demonstrate that mammalian blood vessels, including those of the rabbit's ear, contain an active mono-amine oxidase capable of oxidizing tyramine, adrenaline and noradrenaline. The evidence for this conclusion is based on (1) measurements of oxygen consumption, (2) measurements of ammonia production, and (3) the effect of recognized inhibitors of this enzyme on the oxidation of tyramine.

The significance of the differences in the levels of activity possessed by different blood vessels of the same animal cannot be assessed on our present evidence; it would be of interest, however, to know whether the level of enzymic activity

bears any relation to the sensitivity of different vessels to circulating adrenaline or noradrenaline. Further work on the distribution and properties of this enzyme in the blood vessels is now in progress.

SUMMARY

1. Mono-amine oxidase activity has been demonstrated and estimated in the aorta, carotid, pulmonary, renal, brachial, femoral and ear arteries and in the great veins of the rabbit.

2. It has also been shown to be present in rat and guinea-pig aorta, and in the small muscular branches of the human popliteal artery, which is the only human blood vessel that we have so far studied.

3. A comparison of the levels of enzymic activity in these different blood vessels shows that the great veins and also the brachial, femoral and ear arteries are less active than the larger arteries (aorta, carotid, pulmonary and renal).

We wish to thank Dr R. T. Grant, F.R.S., for his interest and help in this work, and for providing us with a sample of L-noradrenaline bitartrate. Our thanks are also due to the Central Research Fund of the University of London for a grant for the purchase of some of the manometric equipment, and to Mr R. F. Adams for his skilful technical assistance.

REFERENCES

Bhagvat, K., Blaschko, H. & Richter, D. (1939). *Biochem. J.* **33**, 1338.
Blaschko, H. (1940). *Nature, Lond.*, **145**, 26.
Blaschko, H., Richter, D. & Schlossmann, H. (1937*a*). *Biochem. J.* **31**, 2187.
Blaschko, H., Richter, D. & Schlossmann, H. (1937*b*). *J. Physiol.* **90**, 1.
Burn, J. H. (1932). *J. Pharmacol.* **46**, 75.
Florence, G. & Schapira, G. (1945). *C.R. Soc. Biol., Paris*, **139**, 35.
Gaddum, J. H. & Kwiatkowski, H. (1938). *J. Physiol.* **94**, 87.
Markham, R. (1942). *Biochem. J.* **36**, 790.
Philpot, F. J. (1937). *Biochem. J.* **31**, 856.
Philpot, F. J. (1939). *J. Physiol.* **97**, 301.
Philpot, F. J. & Cantoni, G. (1941). *J. Pharmacol.* **71**, 95.
Pugh, C. E. M. & Quastel, J. H. (1937). *Biochem. J.* **31**, 2306.
Richter, D. & Tingey, A. H. (1939). *J. Physiol.* **97**, 265.
Schapira, G. (1945). *C.R. Soc. Biol., Paris*, **139**, 36.
Schaumann, O. (1928). *Arch. exp. Path. Pharmak.* **138**, 208.
Thompson, R. H. S. & Tickner, A. (1949). *Biochem. J.* **45**, 125.