J. Physiol. (1953) 121, 623–628

CHOLINESTERASE ACTIVITY OF ARTERIES

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(*Received* 3 *March* 1953)

Although the presence of enzymes hydrolysing acetylcholine has been described in a wide variety of mammalian tissues, no report has yet been made, as far as we are aware, of the cholinesterase activity of isolated arteries.

In view of the known actions of acetylcholine and eserine on blood vessels (see Dale, 1933; Grant, Bruce Pearson & Comeau, 1936; Alexander, Elliott & Kirchner, 1940), it is to be expected that certain of the structures in the vessel wall might contain a cholinesterase.

It seemed, therefore, that more direct information on isolated arteries was needed, and in the course of experiments designed to study the problem of the increased sensitivity of denervated blood vessels (Armin, Grant, Thompson & Tickner, 1953) the arteries of the rabbit's ear were dissected out, and were found to hydrolyse acetylcholine readily. Since it is now clear that a number of different enzymes, each capable of hydrolysing choline esters, exist in mammalian tissues (Ord & Thompson, 1950), it was thought desirable to characterize this esterase more fully and to study its distribution in different blood vessels.

METHODS

Materials. Rabbit blood vessels were removed from the body after killing the animals by air embolism. Aortas were removed from rats and guinea-pigs after killing by decapitation. Human material was obtained post-mortem and from amputated limbs.

The rabbit ear arteries were briefly perfused with saline before dissection (see Armin *et al.* 1953). All other vessels were well washed with saline *in vitro*, and any blood clots removed; adherent fat and connective tissue which had not been removed from the vessels while in the body were dissected away.

The tissue was then finely minced with scissors, and weighed samples of the mince used for cholinesterase estimation.

Estimation of esterase activity. Esterase activity was determined manometrically at 38° C and at pH 7·4 using the Warburg technique (Ammon, 1933). All estimations were made in duplicate, and were corrected for non-enzymic hydrolysis of the substrate. Activity is expressed as μl.CO_2/g tissue (wet wt.)/hr.

Substrates. (1) Acetylcholine chloride (ACh) (British Drug Houses, Ltd.). (2) Butyrylcholine

perchlorate (BuCh), prepared by Dr G. R. Webster in this laboratory (Earl, Thompson & Webster, 1953). (3) Acetyl-β-methylcholine chloride (MCh) (Savory & Moore, Ltd.). (4) Propionylcholine perchlorate (PrCh), prepared by Dr G. R. Webster. (5) Tributyrin (TB) (British Drug Houses, Ltd.).

The choline esters were dissolved in 0·025 M-$NaHCO_3$ immediately before use to give a final concentration of 0·015 M for ACh and 0·03 M for BuCh, MCh and PrCh. The tributyrin was pipetted directly into the side-arm of the Warburg flasks (0·03 ml. per flask) and was covered with 0·17 ml. of 0·025 M-$NaHCO_3$.

Inhibitors. (1) Eserine sulphate (British Drug Houses Ltd.). (2) Di*iso*propyl fluorophosphonate (DFP).

The inhibitors were dissolved in 0·025 M-$NaHCO_3$ immediately before use.

RESULTS

Preliminary experiments with the isolated central artery of the rabbit's ear showed that acetylcholine is hydrolysed at a rapid rate by this tissue. The aorta and several other large arteries in the rabbit were next studied, and were also found to contain an acetylcholine-hydrolysing enzyme. Although a number of different types of cholinesterase have now been recognized in the tissues of different mammalian species, many of them can still usefully be classified as being either a 'true' cholinesterase or a 'pseudo' cholinesterase (Mendel & Rudney, 1943). To determine whether the activity present in rabbit blood vessels is due to an enzyme falling into one or other of these two main types, we studied the hydrolysis of a number of different choline esters, including acetyl-β-methylcholine as a selective substrate for the true cholinesterase (Mendel, Mundell & Rudney, 1943) and butyrylcholine for the pseudocholinesterase (Nachmansohn & Rothenberg, 1945).

TABLE 1. Hydrolysis rates of different esters by arterial cholinesterases
(Expressed as μl. CO_2/g/hr; no. of experiments in brackets)

Tissue	ACh	BuCh	PrCh	MCh	TB	BuCh / ACh	MCh / ACh
Rabbit aorta	1,875 (7)	5,565 (6)	2,920 (3)	130 (4)	1,880 (5)	3·0	0·07
Rabbit carotid	915 (1)	2,350 (1)	—	212 (1)	4,260 (1)	2·6	0·23
Rabbit brachial	869 (1)	2,166 (6)	—	146 (2)	—	2·5	0·17
Rat aorta	14,250 (2)	19,810 (5)	22,700 (3)	965 (2)	—	1·4	0·07
Guinea-pig aorta	1,630 (2)	5,350 (2)	—	415 (2)	—	3·3	0·25
Human aorta	200 (1)	515 (1)	—	70 (1)	—	2·6	0·35

Table 1 shows the rates at which the different substrates are hydrolysed by various rabbit, rat, guinea-pig and human arteries. The human aorta was obtained from a male aged 42. It will be seen that, except for rat aorta, BuCh is hydrolysed about $2\frac{1}{2}$–3 times as rapidly as ACh, whereas MCh is hydrolysed at about a quarter of the rate of ACh or less by rabbit, rat and guinea-pig arteries, and at about one-third of the rate of ACh by the human aorta. The BuCh/ACh ratio of 1·4 for rat aorta is lower than that obtained for the other species, but agrees well with the ratio of 1·4 obtained for the pseudo-cholinesterase of rat heart by Ord & Thompson (1951). The MCh/ACh ratio varies

rather widely among these different arteries, but with the low levels of activity found in the presence of MCh the values given for the hydrolysis rate of this substrate are likely to be only approximate.

These findings indicate therefore that the cholinesterase in these blood vessels is largely, but not entirely, a pseudo-cholinesterase.

Table 1 also shows that tributyrin is rapidly hydrolysed by rabbit aorta and carotid artery. The fact that the carotid artery splits tributyrin about 4 times as fast as the aorta, relative to the acetylcholine rates for these tissues, suggests that there is present, in addition to the cholinesterase, an ali-esterase which is relatively more plentiful in the carotid artery than in the aorta.

Summation experiments in which the rate of hydrolysis of mixtures of tributyrin and a choline ester are compared with the rates obtained with each of these substrates alone confirm the interpretation that two enzymes are present (Table 2). This is further borne out when the effect of eserine is studied

TABLE 2. Hydrolysis rates of mixtures of tributyrin and choline esters by rabbit arteries (Expressed as μl. CO_2/g/hr)

			Choline ester + TB		
Tissue	ACh	TB	Found	Calc.	'Summation' (%)
Aorta	2730	2760	6040	5490	110
Aorta	1080	1450	2140	2530	84
	BuCh				
Renal	1710	860	2910	2570	113

TABLE 3. Effect of eserine (10^{-7} M) on the hydrolysis of choline esters and tributyrin by arteries

	% inhibition	
Tissue	ACh	TB
Rabbit aorta	59	20
	BuCh	
Rabbit aorta	54	28
Human carotid	47	5

on the hydrolysis of these substrates (Table 3), since tributyrin hydrolysis is found to be significantly less eserine-sensitive than is the hydrolysis of acetyl- or butyrylcholine.

The effect of DFP on the hydrolysis of butyrylcholine by rabbit carotid artery and human aorta was also studied; the cholinesterase in these tissues was found to be highly sensitive to inhibition by this compound, concentrations of 7×10^{-9}M and $2{\cdot}5 \times 10^{-9}$M bringing about 50% inhibition in the rabbit and human tissues respectively. These values agree well with the I_{50} concentrations found for the pseudo-cholinesterase in other tissues of other species. Thus, Mackworth & Webb (1948) found an I_{50} concentration of $1{\cdot}2 \times 10^{-9}$M for the pseudo-cholinesterase of horse serum, while Mazur &

Bodanksy (1946) reported I_{50} concentrations of $6{\cdot}3 \times 10^{-9}$M and 2×10^{-8}M for horse and human serum respectively.

The cholinesterase levels of a number of different rabbit arteries are shown in Table 4. In one experiment the superior and inferior venae cavae were

TABLE 4. Cholinesterase levels of rabbit arteries

(Expressed as μl. CO_2/g/hr. Substrate 0·03 M-butyrylcholine perchlorate)

	Aorta	Carotid	Brachial	Femoral	Pulmonary	Renal	Ear
	6970	3200	1290	115	3430	995	870
	4875	4000	2395	350	2765	540	935
	5160	3585	1975	335	3050	845	985
	5370	2410	3220	230	3560	1710	1355
	7005	3855	2395	415	—	—	545
	4010	4005	1720	665	—	—	1185
Mean	5565	3509	2166	352	3201	1022	979

dissected out, but were found to show only negligible activity, amounting to less than 100 μl./g/hr. No activity was detected in the pulmonary vein. Small lengths of coronary artery, dissected from a number of rabbits, gave a level of 2020 μl./g/hr, but little reliance can be placed on the accuracy of this single estimate.

In two experiments rabbit aorta was slit open longitudinally and the inner layer of the vessel wall (amounting to approximately half the total thickness of the vessel wall) was scraped off the remaining outer part; in each experiment the inner layer showed a level of activity significantly higher than the outer, the average levels being 8115 and 5270 μl./g/hr respectively.

A few experiments with arteries from human subjects showed the presence of cholinesterase activity which, as already pointed out, seems to be a pseudocholinesterase like that in the rodent arteries. The values which we have obtained have ranged from 255 μl./g/hr for the digital arteries, removed from an amputated finger in a middle-aged man, to 1310 μl./g/hr for the common iliac artery from an infant 3 days old. We must stress, however, that we have so far examined only a very few human blood vessels.

DISCUSSION

The experiments described above demonstrate a relatively high level of cholinesterase activity in a number of rabbit blood vessels, in the aorta of the rat and guinea-pig, and, in a few preliminary experiments, in human aorta and arteries. In this connexion it is of interest to recall that Schmiterlöw (1948) has recently identified acetylcholine in extracts of arterial wall.

Summation experiments and sensitivity to eserine indicate that the enzyme concerned in the hydrolysis of acetylcholine by these tissues is distinct from the ali-esterase which is also present and which hydrolyses simple aliphatic esters such as tributyrin. Experiments with different choline esters as

substrates indicate that the cholinesterase in these arteries is chiefly a pseudo-cholinesterase, in that BuCh is hydrolysed more rapidly than ACh, while MCh is attacked only at a low rate. Also, the sensitivity of the acetylcholine-hydrolysing activity to inhibition by DFP is of the same order as that of the classical pseudo-cholinesterases in horse and human serum. The activity of the rabbit aorta in the presence of different choline esters suggests that, like certain of the pseudo-cholinesterases in other species, it is a 'butyro-cholinesterase' (Sturge & Whittaker, 1950), since the rates of hydrolysis are in the order BuCh > PrCh > ACh > MCh. Rat aorta appears to be very rich in an enzyme which, in this species, appears to be a 'propiono-cholinesterase' similar to the enzyme in rat heart, propionylcholine being hydrolysed more rapidly than butyrylcholine.

The different arteries in the rabbit which we have so far studied show some striking differences in activity; the femoral artery in particular shows a relatively low content of cholinesterase, amounting to less than one-tenth of that in the aorta. The distribution of cholinesterase activity in these arteries differs therefore from that obtained for mono-amine oxidase (Thompson & Tickner, 1951), the level of which in the femoral artery is of the same order as that in the brachial and ear arteries, and amounts to about half the level in the aorta.

We cannot reach any conclusion as to the location of the cholinesterase in the arterial wall, but from our observation that the inner layer of the rabbit aorta shows a higher level of activity than the outer we feel justified in regarding the enzyme as associated with some structure or structures in the arterial wall itself, and not due merely to the inclusion in the material under assay of nerve plexuses and other structures in the adventitia and surrounding tissues. Histochemical studies would be of interest as a possible means of determining more exactly the localization of the enzyme. The observations recorded in the preceding paper (Armin *et al.* 1953) suggest that it is partly associated with the sympathetic nerves to the vessels and partly with sensory nerves.

SUMMARY

1. Cholinesterase activity has been demonstrated in a number of rabbit, rat, guinea-pig and human arteries.

2. Experiments are described indicating that this activity is due chiefly to a pseudo-cholinesterase.

We wish to thank Dr G. R. Webster for the synthesis of butyryl and propionylcholine perchlorates, the Chief Superintendent, Experimental Station, Porton, for providing us with DFP, and Mr R. F. Adams for his skilled technical assistance. 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.

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