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Carbonic Anhydrase Activity in Mammalian Skeletal and Cardiac Muscle

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1. The presence of extravascular carbonic anhydrase activity in skeletal muscle, and its absence from cardiac muscle, were demonstrated in the rat. 2. The activity in skeletal muscle is approximately correlated with the proportion of dark fibres present in the middle fibre bundles.

Measurement of the activity of extravascular carbonic anhydrase in a mammalian tissue is hindered by contamination of the tissue homogenate with carbonic anhydrase (carbonate hydro-lyase, EC 4.2.1.1) from erythrocytes in the blood perfusing that tissue. After attempting to overcome this problem by perfusing the tissue with a saline solution before homogenization, Roughton (1935) detected the presence of small amounts of carbonic anhydrase in aqueous extracts of mammalian muscle. The source of the muscle studied was not specified, and no description was given of the efficiency of removal of material of erythrocytic origin from the muscle by the perfusion technique used. Van Goor (1940) reported greater carbonic anhydrase activity in 'red muscle' (presumably skeletal muscle of tonic function) from rats than in 'striated muscle' (presumably fast-twitch skeletal muscle), but the potential existence of a real difference in extravascular carbonic anhydrase activity between the two types of skeletal muscle was obscured by the presence of a greater quantity of blood in the extract of 'red muscle' than in the extract of 'striated muscle'.

More recently, it has been shown that the CO₂ space of the skeletal-muscle bed in the isolated perfused dog hind limb may be decreased from greater than the water space to similar to the vascular space by the administration of acetazolamide {*N*-[5-(aminosulphonyl)-1,3,4-thiadiazol-2-yl]acetamide} (Zborowska-Sluis *et al.*, 1974). This effect was found to be independent of the presence of erythrocytic carbonic anhydrase in the perfusate, providing indirect evidence of the presence of carbonic anhydrase in an extravascular compartment of the skeletal muscle, and challenging the belief (Maren, 1967) that mammalian muscle does not contain extravascular carbonic anhydrase.

A trace of carbonic anhydrase activity was detected in cardiac muscle by Van Goor (1940) in his studies of tissues from the rat, but a further experiment by Zborowska-Sluis *et al.* (1975) revealed a relatively small decrease in dog myocardial CO₂ space after the

administration of acetazolamide, suggesting that cardiac muscle does not contain extravascular carbonic anhydrase.

The present study was undertaken to re-examine the conclusions drawn from the indirect evidence provided by the work of Zborowska-Sluis *et al.* (1974, 1975) by direct determination of the carbonic anhydrase activity of various muscles in the rat and correction of the results for the presence of erythrocytic carbonic anhydrase contributed by blood trapped in the vascular bed of each muscle at the time of homogenization.

Materials and Methods

In the principal experiments 18 albino rats of the Wistar strain, weighing 160–420 g, were used. Each rat was killed with a blow to the neck, then duplicate samples of blood were taken by cardiac puncture, and samples of muscle obtained by rapid dissection. The muscles studied were left ventricular cardiac muscle, vastus lateralis, extensor digitorum longus and soleus. Samples of each type of skeletal muscle obtained from one rat were weighed with a torsion balance, then pooled to obtain sufficient material (150–200 mg wet wt. of tissue) for subsequent determinations. All further procedures were performed at or near 0°C.

Blood samples were haemolysed by addition of water, and further diluted with 5.0 ml of Triton X-100 (BDH Chemicals, Poole, Dorset, U.K.)/litre. Muscles were disrupted in Triton X-100 (5.0 ml/litre) with a motor-driven Teflon–glass Potter–Elvehjem homogenizer, then centrifuged for 60 min at 1500g (r_{av} , 15 cm). Carbonic anhydrase activity was determined in the supernatants of the homogenized muscles and in the diluted haemolysates at 0°C by a modification of the electrometric technique of Wilbur & Anderson (1948). The assay medium comprised 3.0 mM-sodium 5,5'-diethylbarbiturate/0.6 mM-HCl, pH 8.4, with 0.1 g of peptone (Oxoid, London SE1 9HF, U.K.)/litre to ensure that the initial velocity of enzymic hydration of

CO₂ was a function of the enzyme concentration (McIntosh, 1968). The activity of carbonic anhydrase was expressed as enzyme units per g of original tissue; 1 enzyme unit = $(t_0 - t)/t$, where t_0 was the time for the uncatalysed reaction to bring about a change in pH of the assay medium from 8.0 to 6.3, and t was the time for the enzymically catalysed reaction to achieve the same effect. The potential effect on the activity of carbonic anhydrase of the protein inhibitor present in rat serum (Booth, 1938) was considered negligible, as the samples were diluted before assay, and the assays were performed at 0°C.

To estimate the volume of whole blood trapped in each sample of muscle, the principle described by Danielli (1941) was used, the quantity of haemoglobin present in haemolysates and homogenate supernatants being determined by measurement of A_{577} . In a further eight rats (body wt. 175–300 g) the volume of whole blood trapped in each muscle was again estimated by the Danielli (1941) technique, but [¹³¹I]iodinated human serum albumin (The Radiochemical Centre, Amersham, Bucks., U.K.) was used as an alternative indicator to haemoglobin. Each rat was lightly anaesthetized with diethyl ether, and approx. 0.2 ml of a solution of [¹³¹I]iodinated albumin (100 µCi/ml) in 0.9% NaCl was introduced into the tail vein with a hypodermic syringe. After recovering consciousness for 5 min, the rat was killed with a blow to the neck, and samples of whole blood and muscle were taken for counting of their ¹³¹I contents in a crystal-scintillation detector linked to an SR3 spectrometer (Nuclear Enterprises, Reading, Berks., U.K.).

An estimate of the erythrocytic contribution of carbonic anhydrase activity in each muscle homogenate was obtained from the product of the calculated volume of whole blood trapped in the homogenate and the carbonic anhydrase activity determined in blood sampled from the same animal. The

extravascular carbonic anhydrase activity of each muscle was calculated as the difference between the erythrocytic contribution and the total carbonic anhydrase activity determined in the homogenate.

Results and Discussion

Estimated volumes of blood trapped in each type of muscle at the time of homogenization are reported in Table 1. Application of Student's *t* test revealed no statistically significant difference ($P > 0.05$) for any one type of muscle between the mean of the estimates obtained by using endogenous haemoglobin as an indicator and the mean of those obtained by using [¹³¹I]iodinated human serum albumin. The estimates of trapped-blood volume that were obtained by measuring haemoglobin were used for calculation of the net or extravascular carbonic anhydrase activity of each homogenate (Table 1).

In all three types of skeletal muscle, but not in cardiac muscle, the mean extravascular carbonic anhydrase activity was significantly greater than zero ($P < 0.001$; a one-tailed test of significance by using Student's *t* distribution), thus supporting the indirect evidence reported by Zborowska-Sluis *et al.* (1974, 1975). It has been suggested (Zborowska-Sluis *et al.*, 1975) that the presence of extravascular carbonic anhydrase in skeletal muscle, and its absence from cardiac muscle, may be related to differences in cell size and vascularity between the two tissues, so that in the myocardium the average distance from an individual fibre to the nearest capillary lumen is sufficiently short for adequate CO₂ transport to take place by simple diffusion, whereas in skeletal muscle the greater cell size and greater intercapillary distances require the presence of extravascular carbonic anhydrase to facilitate the transport of CO₂ from the muscle fibres to the vascular compartment.

Table 1. *Trapped-blood volumes and activities of extravascular carbonic anhydrase in homogenates of rat cardiac and skeletal muscles*

Analytical procedures, conditions of assay and units of carbonic anhydrase activity are as described in the Materials and Methods section. Means $\pm$ S.E.M. are given, with the numbers of independent determinations in parentheses.

Tissue	Volume of blood trapped in homogenate (μ l of whole blood/g of original tissue)		Total carbonic anhydrase activity (units/g of original tissue)	Erythrocytic carbonic anhydrase activity present in muscle (units/g of original tissue)	Extravascular carbonic anhydrase activity (units/g of original tissue)
	[¹³¹ I]Iodinated human serum albumin as indicator	Endogenous haemoglobin as indicator			
Left ventricular myocardium	120 $\pm$ 10 (8)	91 $\pm$ 17 (18)	17.8 $\pm$ 1.9 (18)	17.4 $\pm$ 1.9 (18)	0.42 $\pm$ 0.33 (18)
Vastus lateralis	9 $\pm$ 1 (8)	12 $\pm$ 1 (18)	7.2 $\pm$ 0.6 (18)	2.2 $\pm$ 0.2 (18)	5.0 $\pm$ 0.5 (18)
Extensor digitorum longus	18 $\pm$ 1 (8)	20 $\pm$ 1 (18)	9.3 $\pm$ 0.7 (18)	3.9 $\pm$ 0.4 (18)	5.4 $\pm$ 0.5 (18)
Soleus	77 $\pm$ 8 (8)	82 $\pm$ 6 (18)	27.6 $\pm$ 2.0 (18)	16.7 $\pm$ 1.6 (18)	10.9 $\pm$ 0.8 (18)

The present investigation also revealed considerable differences in extravascular carbonic anhydrase activity among the three different skeletal muscles that were studied, the mean value for soleus being significantly greater ($P < 0.001$; Student's t test for paired comparisons) than the mean value observed in either vastus lateralis or extensor digitorum longus. Muscles having a tonic function, such as soleus, tend to contain a greater proportion of dark fibres than do muscles of phasic function, such as vastus lateralis or extensor digitorum longus (Sréter & Woo, 1963). As dark (or red) muscle fibres are of smaller diameter, and are associated with a more dense capillary network, and greater blood flow per unit weight of tissue, than light (or white) muscle fibres (Hudlicka, 1973), it would not be appropriate to invoke differences in vascularity to account for the differences in extravascular carbonic anhydrase activity between soleus and the other types of skeletal muscle observed in the present investigation. Most mammalian skeletal muscles contain both dark and light fibres in varying ratios (Sréter & Woo, 1963) and, as the different amounts of extravascular carbonic anhydrase activity found in the three skeletal muscles in this study correlate approximately with the percentage of dark fibres present in the middle fibre bundles of each muscle (vastus lateralis, 36.5%; extensor digitorum longus, 43.7%; soleus, 61.5%) reported by Sréter & Woo (1963), it seems possible that the extravascular carbonic anhydrase present in rat

skeletal muscle may be largely confined to the dark fibres.

The presence of carbonic anhydrase in the extravascular compartment of dark muscle fibres would probably facilitate the transport to the vascular compartment of CO_2 , which is produced in greater quantities by dark fibres, because they have greater capacities for the oxidation of glucose and fatty acids, greater O_2 consumption and lesser activities of glycolytic enzymes in comparison with light fibres (Hudlicka, 1973).

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