Calcium- and anoxia-induced damage of cardiac myocytes in culture

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Tissue necrosis may be detected by the appearance of intracellular enzymes in extracellular spaces (Kibe & Nilsson, 1967) and this principle has been applied to ischaemic and hypoxic cardiac necrosis in man and animals (Roe & Starmer, 1975; Shell et al., 1973). Beating myocardial cells in culture represent an extremely reproducible preparation for studies of the deleterious effect of hypoxia on cardiac tissue. The aim of the study reported here was to examine the metabolic basis of the exacerbation of damage caused by increased medium Ca2+ concentration to heart cells subjected to anoxia.

Experimental
Neonatal myocytes were prepared from 3-day-old rats and cultured as described previously (Higgins et al., 1979). Spent medium was aspirated and replaced with normoxic or anoxic medium (depleted of O2 by purging with O2-free N2) in which the Ca2+ content was adjusted during its preparation. Cultures were incubated for 16 h at 37°C except where time-course studies were performed.

Results and discussion
Fig. 1 shows the effect of increasing medium Ca2+ concentration on lactate dehydrogenase release from anoxic and normoxic cultures. The data show a deleterious effect of Ca2+ on membrane integrity under anoxic conditions. Production of lactate by anoxic cultures was significantly greater than under normoxic conditions.

The time course of enzyme leakage from normoxic and anoxic cultures was examined in the presence of elevated (4.4 mM) and normal (1.9 mM) [Ca2+]. Release of lactate dehydrogenase from normoxic cultures was slight and was not significantly increased by elevation of [Ca2+]. Under conditions of anoxia marked enzyme release was observed, and the rate of enzyme release was increased in the presence of increased [Ca2+]. The anoxia-stimulated increase in rate of lactate production was not affected by the [Ca2+] of the medium.

The deleterious effect of Ca2+ on anoxic cultures could be decreased by inclusion in the medium of nifedipine or verapamil. These drugs are 'Ca2+-antagonists' and have a negative inotropic action. The drugs had no effect on enzyme release from normoxic cultures, but it was possible to demonstrate a significant decrease in enzyme release under anoxic conditions. Lactate production by cultures under both anoxic and normoxic conditions was significantly decreased by 0.1 μM-verapamil. Nifedipine at a concentration of 0.1 μM decreased lactate production under anoxic but not under normoxic conditions.

After anoxic insult in the presence of an elevated medium [Ca2+] it was found that the ATP content of the myocyte cultures was significantly decreased. Inclusion of the Ca2+-antagonist (either nifedipine or verapamil) caused some amelioration of the anoxia- and Ca2+-induced decline in the ATP content of the cultures. These drugs had no effect on the ATP content of normoxic cell cultures.

An increase in the extracellular [Ca2+] appears to exacerbate anoxia-induced damage to cardiac myocytes. This exacerbation may be indirect, resulting from an increased inotropic state during hypoxia. Under anoxic conditions the entry of Ca2+ into the cell via the slow phase of the action potential is inhibited (Nayler et al., 1979) and the inotropic state of the cell decreases. Increasing the external [Ca2+] may possibly overcome this (Winegrad & Shanes, 1962) and the [Ca2+] of the cell increase despite the anoxia-mediated inhibition. Increasing inotropic state in the absence of O2 may be expected to cause the intracellular ATP concentrations to decrease and intracellular Ca2+ homeostasis to be lost. Negative inotropic agents such as verapamil or nifedipine, by decreasing the inotropic state of the cells may decrease ATP utilization and thereby permit salvage of muscle cells.


Unusual kinetic behaviour of plasmin (fibrinolysin) digestion of fibrin and N-benzoxycarbonyltyrosine nitrophenyl ester

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The trypsin-like enzyme plasmin exhibits non-linear product formation with increasing enzyme concentration when assayed as an esterase with N-benzoxycarbonyltyrosine nitrophenyl ester and as a proteinase with fibrin as substrate. The kinetics were typical of substrate inhibition and we wish to draw attention to this probable source of error when assaying plasmin concentrations.

The enzyme plasmin or fibrinolysin is similar to trypsin in many of its properties, yet differs markedly from other...
Plasmin

Fig. 1. Esterase activity of plasmin assayed with Cbz-Tyr-ONp by the release of p-nitrophenol after 25 min at 30°C (curve A) with 33 μg of Cbz-Tyr-ONp/3 ml reaction mixture and (curve B) with 73 μg of Cbz-Tyr-ONp/3 ml reaction mixture.

trypsin-like enzymes in exhibiting non-linear product formation with increasing enzyme concentration when assayed as a proteinase with fibrin and as an esterase with Cbz-Tyr-ONp. We wish to draw attention to this difference, since the assay of plasmin with these substrates must provide values that are too low. In the present study we employed Sigma bovine plasmin (0.45 units/mg).

In Fig. 1, we employed the Cbz-Tyr-ONp assay of Martin et al. (1959) with incremental additions of plasmin (0-10 μg/tube) and 33 μg of Cbz-Tyr-ONp in each tube in Fig. 1, curve A and with 73 μg in each tube in Fig. 1, curve B. The incubation time was 25 min at 30°C, much more than would be used for trypsin or chymotrypsin, which attacked this substrate at much faster rates than plasmin.

It can be seen that the plots exhibit a lag region during which no products were formed from the initial addition of the enzyme until such a concentration was exceeded, when a linear rate of product formation was observed. This lag region was extended by increasing the concentration of substrate, as indicated in the changes observed between Fig. 1, curve A and Fig. 1, curve B. Similar results were obtained when fibrin was used as substrate.

This lag region may be explained by substrate inhibition, which can be demonstrated as a non-linear Lineweaver-Burk plot (see Fig. 2) employing Cbz-Tyr-ONp as substrate.

Since these observations have been made with two different substrates, the protein polymer fibrin and the synthetic ester Cbz-Tyr-ONp, we believe this unusual behaviour is a property of the enzyme fibrinolysin or plasmin. We suggest that care be used to assay plasmin, for this unusual behaviour would not be detected unless some form of incremental analysis of the type presented in Fig. 1 is employed.

* Abbreviation: Cbz-Tyr-ONp, N-benzyloxycarbonyl-tyrosine nitrophenyl ester.

Fig. 2. Lineweaver-Burk plot of esterase activity of plasmin assayed with increasing concentrations of Cbz-Tyr-ONp and a fixed amount (10 μg) plasmin and with 1S in μM.

The functional development of the pathway for steroid hormone production in the adrenal of the foetal sheep

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In foetal sheep, as in the foetuses of several other species (Liggins et al., 1973, 1977), there is a large increase in plasma cortisol or corticosterone concentration (Fig. 1a) before birth that has been ascribed to increased activity of the foetal adrenal gland (Beitins et al., 1970; Nathanielsz et al., 1972; Liggins et al., 1973). The reason for this has not been apparent, as the plasma corticotropin concentration does not rise at this time (Rees et al., 1975; Jones et al., 1977). Despite this, it has still been accepted that the functional activity of the pathway for adrenal steroid hormone secretion increases dramatically over the final days before birth, and earlier studies with adrenal homogenates or slices supported this view (Anderson et al., 1972; Madill & Bassett, 1973).

However, recent experiments with cells prepared from the foetal sheep adrenal by collagenase digestion (Jones & Roebuck, 1980) dispute this view (Fig. 1b). Cells prepared from foetal...