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Wash-out of noradrenaline and its metabolites by calcium-free reperfusion after ischaemia: support for the concept of ischaemia-induced noradrenaline release

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The outflow of noradrenaline (NA) and its metabolites on reperfusion after 60 min of regional ischaemia in the isolated heart of the rat was not diminished when calcium was omitted from the reperfusion medium. The findings suggest a release of NA during ischaemia, washed-out at reperfusion.

Introduction Recently we described the appearance, on reperfusion of rat isolated hearts following prolonged ischaemia, of much higher amounts of noradrenaline (NA) and metabolites when lactate was used as substrate instead of glucose (Abrahamsson *et al.*, 1983). In spite of a detailed analysis of the outflow of NA and its metabolites during and after ischaemia of different degrees and time-periods (Carlsson *et al.*, 1983a;b), the possibility could not be excluded that a significant part of the NA release was induced by the reperfusion *per se*. As a major part of the acute cellular damage produced in ischaemic hearts at reperfusion is calcium-dependent (Hearse *et al.*, 1978; Shine & Douglas, 1983), exclusion of calcium during the reperfusion would be expected to result in a reduction of reperfusion-induced NA release but hardly affect wash-out of NA released during the ischaemic period.

Methods Male Wistar rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.) and the hearts were prepared for ligation of the left coronary vessels and perfused by the method of Langendorff as previously described (Abrahamsson *et al.*, 1983). A Krebs-Henseleit bicarbonate buffer was used for the perfusion and either 11.1 mM glucose (insulin-free medium) or 5.0 mM sodium lactate was used as substrate. After 15 min of equilibration perfusion, the hearts were labelled with 1.1×10^{-8} M (−)-[2,5,6-^{3}H]-NA (49.1–53.5 Ci $mmol^{-1}$) for 35 min and subsequently washed out with amine-free buffer for a period of 40 min. Ischaemia was initiated by ligation of the left coronary vessels and immediately after the ischaemic period (60 min) the hearts were reperfused for 15 min with either normal or calcium-free buffer (0.5 mM disodium edetate added). In some experiments (lactate as substrate) the ligature was not tightened and 120 min after the labelling with [^{3}H]-NA the hearts were subjected to 15 min of calcium-free perfusion followed by 5 min of perfusion with normal buffer (2.6 mmol l^{-1} calcium). During the experimental period coronary flow was recorded and samples of coronary effluent were taken and analysed for ^{3}H and [^{3}H]-NA using high performance liquid chromatography and liquid scintillation. The separation of [^{3}H]-NA from its tritiated metabolites was performed by reverse phase liquid chromatography and u.v.-detection (280 nm) and the mobile phase solvent system was a phosphate buffer (pH 2.45). The radioactivity of the samples was determined in a liquid scintillation counter. Corrections were made by the external standard pulse technique. Efficiency of counting was approx. 40%.

Results are presented as means ± s.e.mean. Statistical significance ($P < 0.05$) test was performed using Student's *t* test (paired or unpaired observations).

Results Ligation of the left coronary artery produced a significant reduction in coronary flow. No difference was seen in coronary flow between glucose-perfused (from 7.7 ± 0.4 ml g^{-1} min^{-1} to 3.7 ± 0.3 ml g^{-1} min^{-1}; n = 8, $P < 0.001$) and lactate-perfused (from 8.3 ± 0.5 to 4.1 ± 0.4 ml g^{-1} min^{-1}; $n = 8$, $P < 0.001$) hearts. In non-ligated hearts perfusion with calcium-free buffer tended to increase coronary flow during the first min of calcium depletion (from 7.5 ± 1.0 to 8.5 ± 1.2 ml g^{-1} min^{-1}). Restoration of calcium, on the other hand, caused a significant ($P < 0.05$) reduction in coronary flow (from 8.7 ± 1.2 to 5.6 ± 0.6 ml g^{-1} min^{-1}).

The effect of calcium depletion on the efflux of ^{3}H and [^{3}H]-NA during 5 min of reperfusion of ischaemic isolated hearts is shown in Figure 1. A significantly ($P < 0.05$) higher efflux of ^{3}H was de-

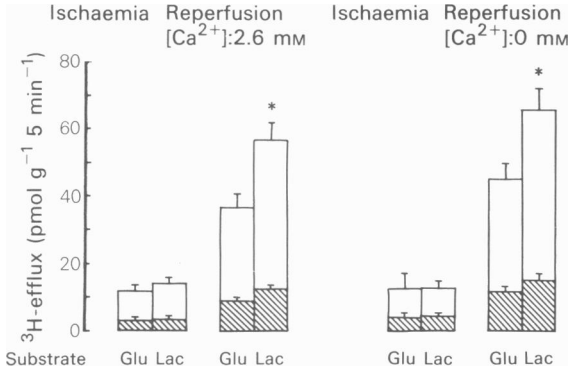

Figure 1. Efflux of ^3H and [^3H]-noradrenaline ([^3H]-NA) during reperfusion after 60 min of left coronary artery ligation in rat isolated perfused hearts. The hearts were perfused with either glucose (Glu) 11.1 mM, or lactate (Lac) 5.0 mM as perfusion substrate. The efflux of total ^3H and [^3H]-NA (hatched area) in the coronary effluent during the last 5 min of ischaemia and during the first 5 min of reperfusion is expressed as pmol g^{-1} (mean ± s.e.; $n=4$). *$P<0.05$ vs. glucose-perfused hearts (Student's t test).

tected from lactate-perfused hearts as than from glucose-perfused hearts both during reperfusion with normal medium and with calcium-depleted buffer. Omission of calcium during the reperfusion did not decrease the outflow of ^3H or [^3H]-NA either from glucose-perfused or lactate-perfused hearts. If anything, there was a tendency for an increased outflow during calcium-free reperfusion.

Calcium depletion in non-ligated hearts (perfusion substrate: lactate 5.0 mM; $n=4$) caused a gradual increase in the efflux of both total ^3H and [^3H]-NA. After 5 min of calcium-free perfusion both total ^3H (from 1.0 ± 0.1 to 3.8 ± 0.2 pmol g^{-1} min^{-1}; $P<0.001$) and [^3H]-NA (from 0.2 ± 0.02 to 0.9 ± 0.2 pmol g^{-1} min^{-1}; $P<0.05$) were significantly increased in the coronary effluent. The total efflux of ^3H was 13.6 ± 0.9 pmol g^{-1} and of [^3H]-NA 3.4 ± 0.8 pmol g^{-1} during the first 5 min.

After 15 min of calcium-free perfusion, restitution of calcium led to a significant ($P<0.01$) increase in the efflux of both [^3H]-NA and total ^3H. During the first min [^3H]-NA increased from 2.0 ± 0.3 to 4.9 ± 0.6 pmol g^{-1} min^{-1} and total ^3H increased from 5.6 ± 0.7 to 8.0 ± 0.9 pmol g^{-1} min^{-1}. The total outflow of ^3H was 33.5 ± 3.8 pmol g^{-1} and of [^3H]-NA 17.4 ± 2.7 pmol g^{-1} during the repletion period (5 min).

Discussion In agreement with a previous report (Abrahamsson *et al.*, 1983) reperfusion of the LAD after 60 min of occlusion in rat isolated perfused hearts was accompanied by a marked outflow of NA and metabolites in the perfusate. Omission of calcium from the perfusion medium did not abolish this increased outflow of catecholamine and metabolites, in fact there was a tendency to an increased outflow. A possible reperfusion-induced release of NA could be the consequence of cellular damage caused by calcium inflow at reperfusion after ischaemia (Hearse *et al.*, 1978). Since low calcium reperfusion of ischaemic myocardium is accompanied by a much better functional and biochemical recovery than reperfusion with a normal calcium concentration (Shine & Douglas, 1983), calcium-free reperfusion in the present experiments was expected to reduce or abolish a reperfusion-induced release of NA from the myocardium. The present results could thus indicate that the total increase in outflow seen at 'normal' reperfusion represents a wash-out of [^3H]-NA and metabolites accumulated during the ischaemic period.

Calcium-free perfusion of the normal myocardium did in fact *per se* induce a certain outflow of NA and metabolites in the perfusate. This finding agrees with earlier reports of the importance of calcium ions for amine retention in rat ventricular slices (Gillis & Paton, 1967; Bogdanski & Brodie, 1969). The outflow of tritium induced by 15 min of calcium-free perfusion of the normal myocardium is quantitatively similar to the additional outflow of tritium found after calcium-free reperfusion as compared with the outflow after reperfusion with normal buffer.

Restoration of calcium after 15 min of calcium-free perfusion of normal myocardium results in a sharp increase in tritium outflow. This increased outflow with calcium restoration is about 30% of that following reperfusion after ischaemia. Thus, the possibility cannot be excluded that such a 'calcium paradox-related' release of NA contributes to some extent to the outflow of NA and metabolites at reperfusion of the ischaemic myocardium; clearly however, the major part of this outflow must be ischaemia-induced.

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