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Extracellular K^+ and protection against cellular damage in the rat heart

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Abstract

Rat hearts were perfused at 37°C with three clearly-defined protocols: the Ca^{2+} paradox, the O_2 paradox and with 20 mM caffeine. Each protocol involved an initial priming (stage 1) and a subsequent full activation (stage 2) of the damage system of the sarcolemma. Raising $[K^+]_o$ from 5.4 to 6.5 mM in the Ca^{2+} paradox had no significant effect, but creatine kinase release was significantly inhibited ($P < 0.001$) at 7.5, 10.8 or 16.2 mM. Raising $[K^+]_o$ to 16.2 mM only during stage 1 or only during stage 2 also inhibited creatine kinase release ($P < 0.001$); protection was greater than when 16.2 mM $[K^+]_o$ was present throughout. $[K^+]_o$ at 10.8 mM exacerbated creatine kinase release in the O_2 paradox ($P < 0.001$) and also when present only during stage 1. However, significant protection was provided when $[K^+]_o$ was raised only during stage 2 ($P < 0.001$). Creatine kinase release in the caffeine protocol was significantly inhibited ($P < 0.001$) at 10.8 mM $[K^+]_o$ and when $[K^+]_o$ was raised only during stage 1 or stage 2. It is concluded that raised $[K^+]_o$ has two opposing effects: prevention of the activation of the membrane damage system and an exacerbation of damage via an increased Ca^{2+} influx.

Keywords: Ca^{2+} paradox; Oxygen paradox; Caffeine; Extracellular K^+; Antiporter

1. Introduction

The Ca^{2+} and O_2 paradoxes are familiar protocols in experimental cardiac physiology and both have been studied in considerable detail (Hearse et al., 1978), although there is wide disagreement concerning the sequence of underlying cellular events that lead to severe damage (Hess and Manson, 1984; Chapman and Tunstall, 1987; Chapman et al., 1991; Altschuld et al., 1991). The two major signs of overt damage are the release (or escape) of cytosolic proteins and the ultrastructural degradation of the myofilament apparatus; these two damage pathways are separate and can be independently activated (Daniels and Duncan, 1993a,1995a). Although there are obvious differences between the two paradoxes, they have characteristic features in common (Hearse et al., 1978), suggesting that the same underlying damage mechanisms are activated in both (Daniels and Duncan, 1995b). Perfusion of the isolated rat heart with caffeine also rapidly causes the release of creatine kinase, but only when initially perfused with Ca^{2+}-free saline (Vander Heide et al., 1986; Vander Heide and Ganote, 1985; Daniels and Duncan, 1993b) so that this experimental protocol has obvious parallels with the Ca^{2+} paradox. Since the system responsible for creatine kinase release can be activated by Ca_o^{2+} depletion (Ca^{2+} paradox; caffeine) or by raised $[Ca^{2+}]_i$ (O_2 paradox) it is suggested to be a transmembrane molecular complex (Duncan, 1990). There is general agreement that a raised $[Ca^{2+}]_i$ is critically implicated in the damage pathways that culminate in the release of creatine kinase and it was initially supposed that a modest depolarization would augment Ca^{2+} entry and so exacerbate damage. However, it has been shown that small rises in $[K^+]_o$ can protect the perfused rat heart in the caffeine protocol (Vander Heide et al., 1986; Vander Heide and Ganote, 1985) and this comparative study was described to test the effects of raised extracellular $[K^+]_o$ in three standardised experimental protocols in which sarcolemma damage is produced by different pathways but all finally act on the same transmembrane damage complex. We show that K^+ has two opposing effects on the initiation of cellular damage in the isolated rat heart. These three experimental techniques are used merely as tools to

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allow an understanding of how $[K^+]_o$ may protect the heart from some forms of dysfunction.

2. Materials and methods

Wistar rats (250–400 g) were lightly anaesthetized in ether prior to intraperitoneal injection of heparin (1000 U/kg) and were killed by cervical dislocation 20 min later. The heart was dissected out, transferred to ice-cold perfusion medium and then cannulated and mounted in a jacketed chamber maintained at 37°C. The isolated heart was perfused using a Watson and Marlow 503S peristaltic pump to maintain a constant flow rate of 8–10 ml/min. The three limbs of the perfusion apparatus allowed rapid change from one medium to another and a preheating coil in each limb immediately above the heart had a bubble trap and with an overflow that was normally held at 40 cm pressure head. A stainless steel heart clip was attached to the base of the left ventricle and linked by a length of cotton to a Biosciences D1 isometric transducer and a Washington MDI oscillograph. The heart was under a tension of 12 g. Mechanical activity was recorded simply to ensure normal contractile activity; any preparations that showed abnormalities or developed an impairment of their mechanical activity during an initial 10 min wash-out and equilibration period were discarded. Coronary flow was measured throughout the perfusion by collection of the coronary effluent at measured intervals and the data were used for the calculation of the rates of creatine kinase efflux.

Hearts were perfused with Krebs-Henseleit medium which contained (mM) NaCl 117, KCl 5.4, $NaHCO_3$ 25, $MgCl_2$ 1, $CaCl_2$ 2.0, NaH_2PO_4 1, glucose 11 made up in distilled water that had been passed through a deionizing column and which was then heavily gassed with $95\%O_2 + 5\%CO_2$ or $95\%N_2 + 5\%CO_2$ ('anoxic medium') and the pH finally adjusted to 7.4 ± 0.05 if necessary. Nominal Ca^{2+}-free solutions, had $CaCl_2$ omitted without ionic substitution and were briefly stored in plastic containers; all glassware was acid-washed ($[Ca^{2+}] < 10^{-6}$ M); these solutions always primed the hearts satisfactorily in the Ca^{2+} paradox and caffeine protocols. Measurements of creatine kinase activity in samples of coronary effluent were made by the linked assay method of Jones et al. (1983) the production of NADPH being followed at 340 nm.

The standard protocols for the three experimental procedures were:

Ca^{2+} paradox: Initial wash-out and equilibration, 10 min. Ca^{2+} free (stage 1), 3 min. Ca^{2+} repletion (stage 2), 9 min.

O_2 paradox: Initial wash-out and equilibration, 10 min. Anoxic medium with glucose omitted (stage 1), 40 min. Return of O_2 with glucose omitted (stage 2), 18 min.

Caffeine: Initial wash-out and equilibration, 10 min. Ca^{2+} free (stage 1), 3 min. Caffeine (20 mM) in Ca^{2+}-free medium (stage 2), 9 min.

Hearts perfused for 60 min with standard Krebs-Henseleit saline retained normal contractility and did not leak creatine kinase.

The pattern of creatine kinase release over 9 min was sigmoidal and all results were compared over the linear part of the graph by an analysis of covariance program. The program compares linear regression, following Sokal and Rohlf (1981) and the regression statistics are included in the program output. A test of regression slopes precedes a test of elevations. Comparison limits are shown if the regressions differ significantly.

All inorganic salts were AnalaR grade; biochemicals were obtained from Sigma (St. Louis, MO, USA) and hexokinase glucose-6-phosphate dehydrogenase preparation was obtained from Boehringer-Mannheim (Lewes, UK). Experiments carried out under U.K. Home Office project licence number 40/00928.

3. Results

3.1. Ca^{2+} paradox

The cumulative creatine kinase release following Ca^{2+} reperfusion in the standard Ca^{2+} paradox protocol is shown in Fig. 1. No creatine kinase was released when the Ca^{2+} paradox was carried out at 28°C (Fig. 1). Hearts were then perfused with a range of Krebs-Henseleit media in which $[K^+]$ was changed from the standard 5.4 mM to 2.7, 6.5, 7.5, 10.8 or 16.2 mM. When $[K^+]_o$ was raised above 10 mM during washout there was a cessation of contractile

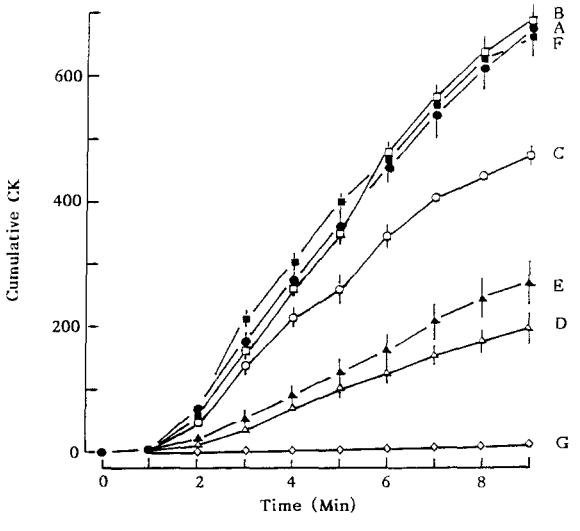

Fig. 1. Effect of $[K^+]_o$ on the Ca^{2+} paradox. In all figures, ordinate shows mean cumulative release of creatine kinase (IU/g dry weight) and abscissa shows duration (min) of stage 2. Vertical lines represent ± S.E.M. (A) Standard Ca^{2+} paradox, $[K^+]_o = 5.4$ mM ($n = 7$). (B) $[K^+]_o = 6.5$ mM ($n = 4$). (C) $[K^+]_o = 7.5$ mM ($n = 4$). (D) $[K^+]_o = 10.8$ mM ($n = 8$). (E) $[K^+]_o = 16.2$ mM ($n = 5$). (F) $[K^+]_o = 2.7$ mM ($n = 3$). (G) standard Ca^{2+} paradox, $[K^+]_o = 5.4$ mM, at 28°C ($n = 3$).

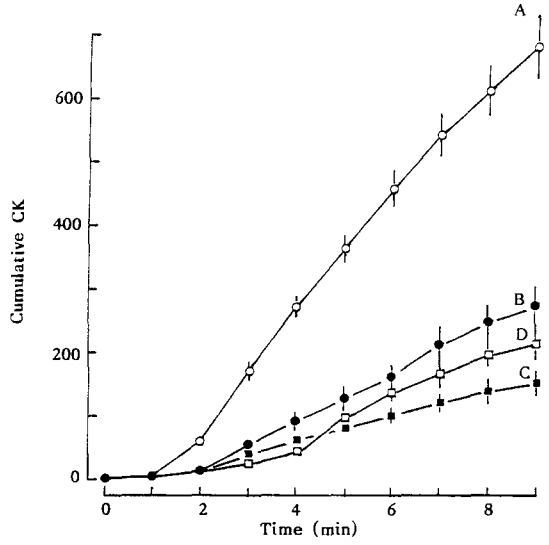

Fig. 2. Effect of 16.2 mM $[K^+]$ on the different stages of the Ca^{2+} paradox. (A) Standard Ca^{2+} paradox, $[K^+]_o = 5.4$ mM ($n = 7$). (B) $[K^+]_o = 16.2$ mM in stages 1 and 2 ($n = 5$). (C) $[K^+]_o = 16.2$ mM in stage 1 and 5.4 mM in stage 2 ($n = 5$). (D) $[K^+]_o = 5.4$ mM in stage 1 and 16.2 mM in stage 2 ($n = 5$).

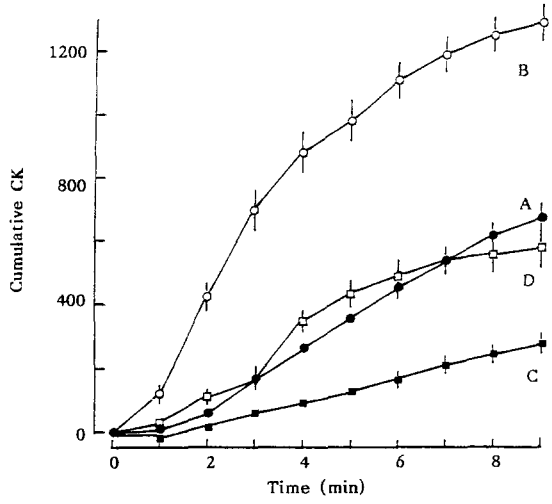

Fig. 3. Interacting effects of raised $[K^+]_o$ and raised perfusion pressure on the Ca^{2+} paradox. (A) $[K^+]_o = 5.4$ mM, pressure = 40 cm water ($n = 7$). (B) $[K^+]_o = 5.4$ mM, pressure = 80 cm water ($n = 6$). (C) $[K^+]_o = 16.2$ mM, pressure = 40 cm water ($n = 5$). (D) $[K^+]_o = 16.2$ mM, pressure = 80 cm water ($n = 6$).

activity and for this reason the change in $[K^+]_o$ was carried out at the start of Ca^{2+} depletion (stage 1). The results are shown in Fig. 1. Raising $[K^+]_o$ from 5.4 to 6.5 mM had no significant effect (Fig. 1). However, total cumulative creatine kinase release was significantly reduced from mean control value of 678 ± 45.3 to 474 ± 17.1 (mean ± S.E.M.) IU/g dry weight ($P < 0.001$) at 7.5 mM $[K^+]_o$. Increasing $[K^+]_o$ to 10.8 mM produced a further significant reduction in cumulative creatine kinase release to 201 ± 22.0 IU/g dry weight ($P < 0.001$). However, when $[K^+]_o$ was further increased to 16.2 mM, the mean cumulative release was 271 ± 36.7 IU creatine kinase/g dry weight which was significantly less than control values at 5.4 mM $[K^+]_o$ ($P < 0.001$) but was significantly greater than the release at 10.8 mM $[K^+]_o$ ($P < 0.01$), see Fig. 1.

When $[K^+]_o$ was reduced to 2.7 mM at 40 cm water pressure, no significant effect on creatine kinase release was found (Fig. 1).

When hearts were perfused with 16.2 mM $[K^+]_o$ only during stage 1 (Ca^{2+} depletion) of the Ca^{2+} paradox, mean cumulative creatine kinase release was 154 ± 21.2 IU/g dry weight (Fig. 2), surprisingly a significantly greater inhibition ($P < 0.001$) than when this $[K^+]_o$ was used in both stages. In a single experiment, the heart was also perfused with 10.8 mM K^+ only during stage 1 and cumulative creatine kinase release was 483 IU/g dry weight, considerable protection against a control paradox with normal $[K^+]_o$ (678 IU/g dry weight) but inhibition was markedly less than with 10.8 mM $[K^+]_o$ throughout the protocol.

Hearts perfused with 16.2 mM $[K^+]_o$ only during Ca^{2+} repletion in stage 2 released a cumulative total of 213 ± 24.7 IU creatine kinase/g dry weight, a significant protection from control values ($P < 0.001$) and, again, a significantly greater inhibition than when 16.2 mM $[K^+]_o$ was present in both stages (271 IU creatine kinase; see Fig. 2). The cumulative creatine kinase released was significantly higher than for 16.2 mM $[K^+]_o$ perfused only during stage 1 ($P < 0.01$).

When the perfusion pressure was raised to 80 cm water during the Ca^{2+} paradox, total cumulative creatine kinase release rose significantly from 678 ± 45.3 (control values) to 1289 ± 60.5 IU/g dry weight ($P < 0.001$; Fig. 3) which was again significantly inhibited when 16.2 mM $[K^+]_o$ was included throughout the protocol, falling to 577 ± 62.4 IU creatine kinase/g dry weight ($P < 0.001$; Fig. 3). Thus 16.2 mM $[K^+]_o$ reduced cumulative creatine kinase release

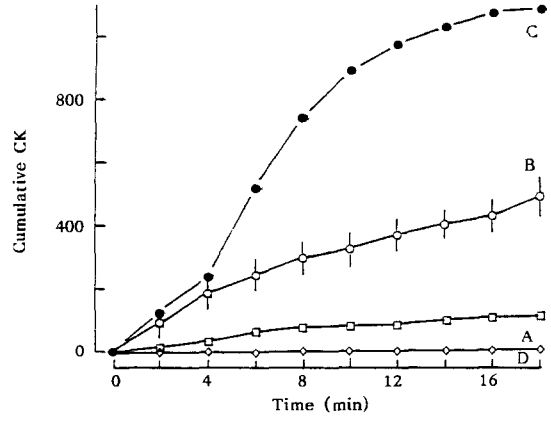

Fig. 4. Effect of the length of anoxic perfusion in stage 1 of the O_2 paradox. (A) 20 min (single experiment). (B) 40 min ($n = 5$). (C) 60 min (single experiment). (D) 40 min at 28°C ($n = 3$). $[K^+]_o$ in all experiments = 5.4 mM.

to 45% at 80 cm compared with 40% at 40 cm water pressure.

3.2. O_2 paradox

In the standard O_2 paradox at 37°C (40 min anoxia, stage 1), with glucose omitted from the perfusion medium, a cumulative total of 444 ± 54.6 IU creatine kinase/g was released during the 18 min reoxygenation in stage 2. In single experiments, 20 min anoxia produced a cumulative total of 117 IU (25%) and 60 min anoxia in stage 1 produced 1097 IU creatine kinase/g dry weight (250%); see Fig. 4. Prolongation of stage 1 progressively increased creatine kinase release in stage 2. No creatine kinase was released when the standard O_2 paradox was carried out at 28°C (Fig. 4).

Raising $[K^+]_o$ from 5.4 to 10.8 mM throughout both stages of the O_2 paradox (40 cm water pressure) resulted in a significant increase in mean cumulative creatine kinase release from 444 to 631 ± 22.8 creatine kinase/g dry weight ($P = 0.01$; Fig. 5), in contrast with the protective effect in the Ca^{2+} paradox. When $[K^+]_o$ was raised to 10.8 mM only during stage 1 cumulative creatine kinase release was also raised to 596 ± 10.1 IU/g dry weight, significantly higher than the control ($P = 0.01$; Fig. 5) but this value did not differ significantly from creatine kinase release when $[K^+]_o$ was raised throughout the paradox (Fig. 5).

Hearts perfused with 10.8 mM $[K^+]_o$ only during stage 2, following perfusion with normal $[K^+]_o$ in stage 1, released a cumulative total of 320 ± 43.2 IU creatine kinase/g dry weight (Fig. 5) which was significantly different from control O_2 paradox results ($P < 0.001$) and from hearts perfused with 10.8 mM $[K^+]$ throughout both

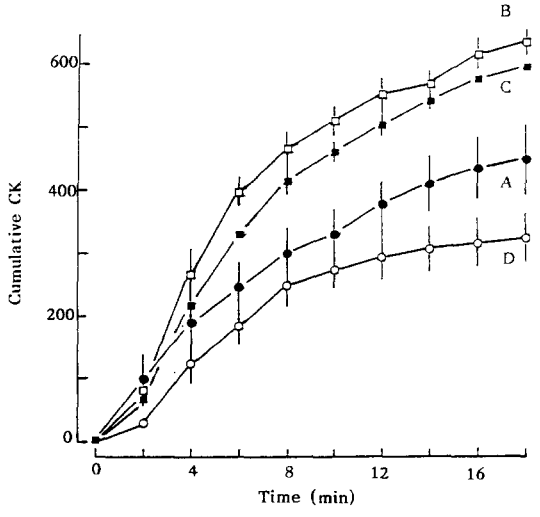

Fig. 5. Effect of raised $[K^+]_o$ on the O_2 paradox. (A) standard O_2 paradox, $[K^+]_o = 5.4$ mM ($n = 5$). (B) $[K^+]_o = 10.8$ mM both in stages 1 and 2 ($n = 5$). (C) $[K^+]_o = 10.8$ mM in stage 1 and 5.4 mM in stage 2 ($n = 3$). (D) $[K^+]_o = 5.4$ mM in stage 1 and 10.8 mM in stage 2 ($n = 3$).

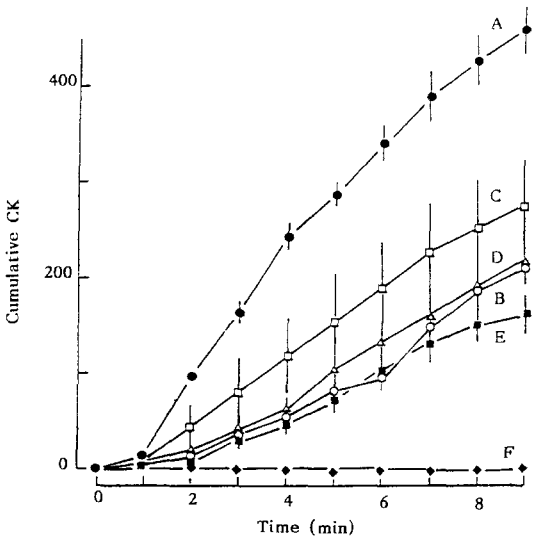

Fig. 6. Effect of raised $[K^+]_o$ on creatine kinase release during the caffeine protocol. Caffeine concentration = 20 mM. (A) Standard, $[K^+]_o = 5.4$ mM ($n = 5$). (B) $[K^+]_o = 10.8$ mM in stages 1 and 2 ($n = 4$). (C) $[K^+]_o = 10.8$ mM in stage 1 and 5.4 mM in stage 2 ($n = 5$). (D) $[K^+]_o = 5.4$ mM in stage 1 and 10.8 mM in stage 2 ($n = 4$). (E): $[K^+]_o = 5.4$ mM, no Ca^{2+} depletion priming in stage 1 ($n = 3$). (F) $[K^+]_o = 5.4$ mM at 28°C ($n = 3$).

stages ($P < 0.01$) or only during stage 1 ($P < 0.01$). In summary: raised $[K^+]_o$ in both stages or only during stage 1 exacerbated creatine kinase release whereas raised $[K^+]_o$ only in stage 2 provided a small but significant protection of the heart.

3.3. Caffeine protocol

Hearts were perfused at a constant flow of 8–10 ml min^{-1} at 37°C with the air bleed closed with Ca^{2+}-free saline for 3 min (stage 1) before perfusion with Ca^{2+}-free saline containing 20 mM caffeine (stage 2). Total cumulative creatine kinase release was 458 ± 27.7 IU/g dry weight (Fig. 6). Omission of stage 1 (i.e., perfusion of caffeine in Ca^{2+}-free saline with no Ca^{2+}-free priming) significantly reduced cumulative creatine kinase release to only 160 ± 21.8 IU/g dry weight ($P < 0.001$), Fig. 6, showing the importance of initial priming as in the Ca^{2+} paradox. Creatine kinase release was completely inhibited when the perfusion temperature was reduced to 28°C (Fig. 6).

When $[K^+]_o$ was raised to 10.8 mM throughout both stages of the caffeine protocol, creatine kinase release was reduced from 458 to 214 ± 18.4 IU/g dry weight ($P < 0.001$; Fig. 6). When $[K^+]_o$ was raised to 10.8 mM only during stage 1, cumulative creatine kinase release was again significantly reduced to 273 ± 56.2 IU/g dry weight ($P < 0.01$; Fig. 6). Raising $[K^+]_o$ only during stage 2 also significantly protected, with creatine kinase release reduced to 212 ± 21.7 IU/g dry weight ($P < 0.001$; Fig. 6).

The results show that raising $[K^+]_o$ in either of the stages alone is almost as effective in protecting as raising $[K^+]_o$ throughout the caffeine protocol.

4. Discussion

The underlying events in the three experimental protocols are summarised in Table 1 which underlines the basic similarities and differences between them. Caffeine and the Ca^{2+} paradox both require priming via Ca^{2+} depletion in stage 1 and $[Ca^{2+}]_i$ rises in stage 2 (although the Ca^{2+} has different origins). On the other hand, $[Ca^{2+}]_i$ rises in stage 1 of the O_2 paradox because the reduced supply of high energy phosphates results in a progressive loss of Ca^{2+} homeostasis. All three protocols are critically dependent on a rise in $[Ca^{2+}]_i$ and are completely protected by reducing the perfusion temperature in stage 1 to 28°C (Hearse et al., 1978; see Figs. 1, 4 and 6). It is suggested that the same transmembrane molecular complex is responsible for creatine kinase release in the damaged cardiomyocyte in the three protocols and that its activation is critically dependent on temperature-dependent molecular mobility within the bilayer (Duncan, 1990).

Raised $[K^+]_o$ has two opposing effects on creatine kinase release: inhibition (as seen in the Ca^{2+} paradox and caffeine) and exacerbation (stage 1 of the O_2 paradox) of the damage. Inhibition in the Ca^{2+} paradox is 'dose-dependent' in the range 5.4 to 10.8 mM $[K^+]_o$ and, since it is effective when raised only during stage 1, it is concluded that the damage system is inhibited during the initial priming of Ca^{2+} depletion. Raised $[K^+]_o$ when applied only during stage 2 of the Ca^{2+} paradox (when $[Ca^{2+}]_i$ rises, Chapman and Tunstall, 1987) protects almost equally effectively, as when it is raised throughout suggesting that (i) it is acting on the same sarcolemma damage system and preventing its full activation in stage 2 by raised intracellular Ca^{2+} (ii) inhibition is still effective even when the damage system has already been primed by Ca^{2+} depletion in stage 1 and (iii) Ca^{2+} entry or the effect of raised $[Ca^{2+}]_i$ in stage 2 of the Ca^{2+} paradox (Chapman and Tunstall, 1987) is dependent on the continued activity of the primed transmembrane damage system (Fig. 2).

Increasing the perfusion pressure produces a corresponding increase in creatine kinase release during the Ca^{2+} paradox. Apparently the damage system is sensitive to perfusion pressure although the percentage inhibition produced by raised $[K^+]_o$ remains approximately constant (Fig. 3).

Initial Ca^{2+} depletion is obligatory for a Ca^{2+} paradox, but in the caffeine protocol, where caffeine is perfused in Ca^{2+}-free saline during stage 2, a small release of creatine kinase is seen with no Ca^{2+}-free priming (Fig. 6). Raising $[K^+]_o$ to 10.8 mM only during either stage 1 or stage 2 of the caffeine protocol was almost equally effective at inhibiting creatine kinase release as raising $[K^+]_o$ throughout (Fig. 6). Vander Heide et al. (1986) also report that creatine kinase release is reduced with 16 mM $[K^+]_o$ in the caffeine protocol.

Raising $[K^+]_o$ would be expected to cause depolarization and increase the Ca^{2+} permeability of the sarcolemma, so exacerbating a rise in $[Ca^{2+}]_i$. This effect is seen in stage 1 of the O_2 paradox where the damage system is not primed by Ca^{2+} depletion in this protocol. The longer the period of anoxia in stage 1, the greater the rise in $[Ca^{2+}]_i$ with a corresponding increase in creatine kinase release in stage 2 (Fig. 4); raised $[K^+]_o$ in stage 1 also causes a 50% increase in creatine kinase release. Evidently, any inhibition of the initial activation in stage 1 of the O_2 paradox by raised $[K^+]_o$ is completely masked by the exacerbated rise in $[Ca^{2+}]$.

The conflicting effects of raised $[K^+]_o$ are illustrated in the Ca^{2+} paradox where 16.2 mM $[K^+]_o$ has a smaller inhibitory action than 10.8 mM (Fig. 1), suggesting that the increased inhibition with raised $[K^+]_o$ is antagonised by raised Ca^{2+} influx associated with depolarisation. The full complexity of the interacting effects of raised $[K^+]_o$ is illustrated in the results for the Ca^{2+} paradox shown in Fig. 2 where 16.2 mM $[K^+]_o$ present only during stage 1 provided greater protection than when $[K^+]_o$ was raised throughout. It might be suggested that depolarization with this high $[K^+]_o$ promoted Ca^{2+} entry in stage 2, so

Table 1
Summary of suggested sequence of events in the three protocols

Protocol	Stage 1		Stage 2	
	sequence of events	effects of raised $[K^+]_o$	sequence of events	effects of raised $[K^+]_o$
Ca^{2+} paradox	Ca^{2+} depletion → priming of sarcolemma damage system. H^+ efflux and other ion movements	protection	Ca^{2+} repletion → rise in $[Ca^{2+}]_i$, full activation and creatine kinase release	protection
O_2 paradox	anoxia → progressive rise in $[Ca^{2+}]_i$ and priming of damage system	exacerbation of creatine kinase release	reoxygenation → full activation and creatine kinase release	small protection
Caffeine	Ca^{2+} depletion → priming of sarcolemma damage system	protection	caffeine → Ca^{2+} release from intracellular storage → full activation and creatine kinase release	protection

accounting for the reduced protection when $[K^+]_o$ is raised in both stages. However, when $[K^+]_o$ was raised only during stage 2 of the Ca^{2+} paradox (Fig. 2), protection was greater than when $[K^+]_o$ was raised throughout. We conclude that inhibition of the transmembrane damage complex is the major effect of raised $[K^+]_o$ in the Ca^{2+} paradox and that promotion of Ca^{2+} entry is a secondary effect.

Raising $[K^+]_o$ only during stage 2 of the O_2 paradox does not exacerbate creatine kinase release (unlike its effect in stage 1) but has a small inhibitory effect with release falling to 72% of control O_2 paradox values (Fig. 5). Presumably the major effect of raised $[K^+]_o$ in augmenting Ca^{2+} entry via depolarization is during stage 1 and is broadly comparable with a longer period of anoxia (Fig. 4); an increased Ca^{2+} influx in stage 2 has only a modest effect in increasing a further rise in $[Ca^{2+}]_i$ and so exacerbating creatine kinase release. Again, therefore, raised $[K^+]_o$ inhibits the sarcolemma damage system, even at this late stage in the O_2 paradox protocol when the membrane damage system (unlike the Ca^{2+} paradox) is finally fully activated by the return of molecular O_2.

This comparative study of three standardised experimental protocols was designed to produce sarcolemma damage in the perfused rat heart by different underlying pathways, but all acting via the same transmembrane damage complex. Since the isolated heart can be exposed to raised $[K^+]_o$ in each of the two stages separately in each protocol, the experiments give some insight into the events during damage and these are summarised in Table 1. It is suggested that raised $[K^+]_o$ has two opposing effects (i) inhibition of the membrane damage complex in stage 1 of the Ca^{2+} paradox and caffeine protocols and in stage 2 in all three protocols (Table 1) and (ii) promotion of Ca^{2+} entry via depolarization of E_m, so exacerbating the rise in $[Ca^{2+}]_i$ that is integral to cellular damage in the myocyte. As $[K^+]_o$ is progressively raised, the augmented influx of Ca^{2+} becomes increasingly important.

In Ca^{2+}-free solutions ($[Ca^{2+}]_o < 1$ μM) the myocyte membrane depolarizes to about -30 mV (Chapman and Tunstall, 1987) so that the additional depolarizing effects of a small rise in $[K^+]_o$ from 5.4 to 10.8 mM would be marginal and we conclude that the inhibitory (or protective) effect of raised $[K^+]_o$ is not via a depolarization of E_m in stage 1 of the Ca^{2+} paradox or caffeine protocols. Indeed, it is possible that the depolarisation associated with Ca^{2+} depletion in these protocols is the key event in the initial activation of the membrane damage system (Rodrigo and Chapman, 1991), starting the intracellular production of H^+ which are transferred across the sarcolemma via the action of the antiporter (Daniels and Duncan, 1995a). It might be suggested that the protective effect of raised $[K^+]_o$ is via an inhibition of Na_i^+/Ca_o^{2+} exchange which is believed to occur in stage 2 of the Ca^{2+} paradox (Chapman and Tunstall, 1987). However, no such Ca^{2+} entry is possible in the caffeine protocol, nor in stage 1 of the Ca^{2+} paradox which take place in Ca^{2+}-free solutions and where raised $[K^+]_o$ is protective. We conclude that the protective effect of a small rise in $[K^+]_o$ is not via Na^+/Ca^{2+} exchange.

Thus, a small rise in $[K^-]_o$ inhibits the system that promotes creatine kinase release either during priming in stage 1 or during full activation in stage 2. Its action could be (i) prevention of activation (ii) prevention of a fall in pH_i or (iii) indirect or direct inhibition of the Na^+/H^+ antiporter, the protective effect being comparable with that of amiloride in the Ca^{2+} paradox and caffeine protocols (Daniels and Duncan, 1995b).

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