The rate of reactivation of the cardiac sodium hydrogen exchanger following inhibition with cyanide

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The cardiac sodium hydrogen exchanger (NHE1) has been implicated in ischaemia/reperfusion damage of the heart. Coupled activity of NHE1 and the sodium calcium exchanger (NCX) are thought to cause calcium overload which is responsible for the resulting the contractile dysfunction and tissue necrosis (Allen & Xiao, 2003).

It is believed that the NHE1 is inactivated by some aspect of metabolic inhibition that occurs during ischaemia and reactivated upon reperfusion (Lazdunski *et al.*, 1985; Park *et al.*, 1999). The time course of this reactivation has indirectly been shown to be very rapid. Park *et al.* (1999) demonstrated that $[Na^+]_i$ started to rise within 30 seconds of reperfusion and reached a peak after 5 minutes. In this study we directly examined the rate of proton flux (J_H ($mmol \bullet l^{-1} \bullet min^{-1}$)) via NHE1 in the acid-loaded ventricular myocytes.

Female SD rats (4-6 weeks) were anaesthetised with pentobarbitone. Single ventricular myocytes were isolated from the heart using a combination of enzymatic digestion and mechanical dispersion. Cells were loaded with the pH indicator carboxy- SNARF-1 and perfused with bicarbonate-free HEPES buffered solution, conditions under which NHE1 is the only acid-extruding mechanism. An isolated cell was then exposed to an NH_4Cl (20 mM) prepulse, and the rate of recovery from acidosis was measured (dpH_i/dt). After return of pH_i to the resting level (7.1) the cell was exposed for 10 minutes to a 2mM NaCN followed by a second NH_4Cl prepulse. The rate of recovery from acidosis was then assessed in the presence of NaCN and then upon its removal.

In control conditions J_H was 0.086 ± 0.022 mM/min (mean ± SEM) when J_H values were calculated in the pH_i range 6.84 − 7.0. In the presence of 2mM NaCN, the J_H value decreased to 0.017 ± 0.005 ($P < 0.05$). This data shows that cyanide inhibits the exchanger. Within 30 s of removal of NaCN, the proton flux had increased to 0.0151 ± 0.019 but part of this apparent flux is caused by the metabolic changes associated with removal of cyanide. After the correction for the effects of the removal of NaCN the mean J_H value was 0.108 ± 0.022 whereas the control measured over the same pH range was 0.050 ± 0.017 which is significantly smaller. These data suggest that the NHE1 activity rapidly reactivates after removal of metabolic inhibition and may show a period of enhanced activity.

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