Mn^{2+}-Enhanced ^1H_2O MRI Measurement of Na^+/Ca^{2+}-Exchanger Mediated Mn^{2+} Influx in the Rat Heart During Myocardial Hypoxia

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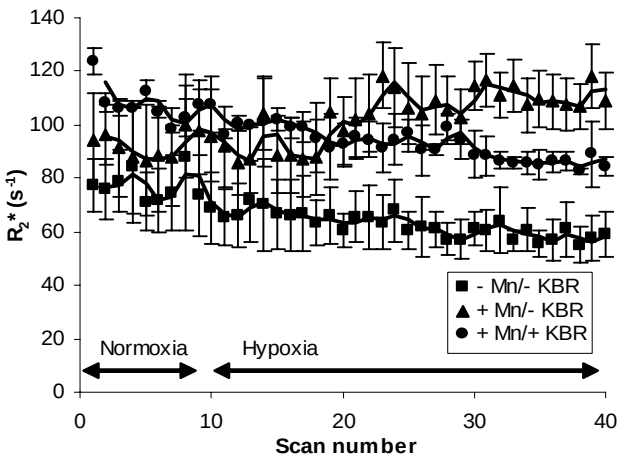
Introduction: Data presented herein support the hypothesis that increases in free intra-cellular (i.c.) calcium, Ca^{2+}, during hypoxia are mediated by the Na^+/Ca^{2+}-exchanger (NCX), which can be blocked with KBR-7943 (KBR), an NCX blocker specific for the reverse-direction[1]. Hypoxia diminishes oxidative metabolism and lowers the i.c. pH as a result of extensive glycolysis. In addition, the hypoxia-induced cellular ATP deficit reduces Na^+/K^+-ATPase activity. In a compensatory attempt to restore cellular homeostasis, myocytes exchange i.c. H^+ for extracellular (e.c.) Na^+, *via* the Na^+/H^+-exchanger. The NCX, operating in "reverse" of the physiological normal net direction, exchanges i.c. Na^+ for e.c. Ca^{2+} leading to a rise in free $[Ca^{2+}]_{i.c.}$. The transmembrane Ca^{2+} influx can be traced with a true Ca^{2+} competitor[2], Mn^{2+}, which is observed *via* its effect on the ^1H_2O MRI signal[3]. The myocardial ^1H_2O R_2 ($\equiv T_2^{-1}$) value *increases* during hypoxia as long as the perfusion flow, and hence the $[Mn^{2+}]_{e.c.}$, is maintained constant—thus allowing for myocardial Mn^{2+} uptake.

Methods: The apparent rate constant, R_2*, is a measure of hyperfine plus susceptibility contributions to transverse relaxation. Values were measured for 9 rat hearts *via* ^1H_2O MRI (AMX 9.4 T Bruker, vertical bore system), before (10 scans) and after (30 scans) the onset of hypoxia, such that each heart served as its own control. The 9 rats were divided into 3 groups of 3 rats each: -Mn^{2+}/-KBR, +Mn^{2+}/-KBR, and +Mn^{2+}/+KBR. The data were acquired with a single 1 mm thick transverse slice using a RARE sequence, $\alpha = 90°$, TR = 12.3 s, TE = 0.023 s, FOV = $(2\text{ cm})^2$, and matrix = $(128)^2$. Spin-density (S_0) and R_2* values were obtained using the *Paravision*® mono-exponential fitting routine. Hearts were Langendorff-perfused[4,5] with a 23°C modified Krebs-Henseleit solution—PO_4 and SO_4 were replaced with Cl^-, and $[K^+]_{e.c.}$ was held at 15.75 mM to arrest the heart. The perfusion pressure was monitored constantly throughout each imaging session. The perfusing medium contained 25 μM Mn^{2+} whenever applicable.

Results: One of the rats was excluded from the group receiving KBR due to an incorrect Mn^{2+} loading of the heart. The mean normalized S_0 values among the 3 groups were not significantly different before or after hypoxia, according to the non-parametric Kruskal-Wallis test for k-independent samples (asymp. p-value = 0.875). The plot below depicts the mean R_2* time-course for each group, before (scans 1 to 10) and after (scans 11 to 40) the onset of hypoxia. The mean R_2* values (in s^{-1}), before and after hypoxia, for each group are: 76.5 (±6.0) and 62.4 (±4.9); 92.7 (±4.7) and 103.9 (±9.9); and 107.9 (±6.7) and 92.6 (±6.1), for the -Mn^{2+}/-KBR (■), +Mn^{2+}/-KBR (▲), and +Mn^{2+}/+KBR (●) groups, respectively. After hypoxia, the mean R_2* values for the control group (■) and that (●) receiving KBR are reduced by 18.4% and 14.2% relative to their respective baseline values. The mean R_2* value for the group (▲) receiving Mn^{2+} but not KBR increased 12.1%, reflecting myocardial Mn^{2+} uptake. These differences are significant to p-value <0.001 according to Tukey and Bonferroni *Post Hoc* tests.

Conclusion: Decrements in control (■) and KBR-loaded (●) mean R_2* values during the experiment reflect cardiac arrest/hypoxia-induced edema. Although S_0 values are expected to increase with edema, these changes were not significant according to the non-parametric Kruskal-Wallis test for k-independent samples. On the other hand, the mean R_2* value for the group (▲) receiving Mn^{2+} but not KBR increases under hypoxia presumably because the rise in free paramagnetic $[Mn^{2+}]_{i.c.}$ catalyzes the loss of NMR coherence among ^1H_2O spins with which Mn^{2+} interacts, thus offsetting the edematous effects. The ability of Mn^{2+} to catalyze ^1H_2O R_2* is strongly dependent on the reorientation time (τ_r) of its complex. For instance, protein-bound Mn^{2+} has a greater relaxivity than the Mn^{2+} aqua ion, because the former has a larger τ_r value.[3] One may expect that increases in free $[Ca^{2+}]_{i.c.}$, and in its tracer Mn^{2+}, would translate into greater or smaller R_2* values depending on whether this rise stems from a Ca^{2+} influx or its release from intracellular populations, respectively. Therefore, the results reported herein are consistent with the hypothesis that increases in $[Ca^{2+}]_{i.c.}$ during hypoxia result from Ca^{2+} influx rather than its release from intracellular protein. [Though unlikely, R_2* value reduction could be accounted for by changes in the heart geometry and increased bulk magnetic susceptibility contributions to R_2* during the experiments.] Since the nifedipine-sensitive slow Ca^{2+}-channel[2,6] is inoperative under hyperkallemic conditions and receptor mediated endocytosis of divalent metal cations[7-9] is a slow process, Mn^{2+} enters the cell likely through the NCX during hypoxia—this is consistent with the observed inhibition of the Mn^{2+} effect on R_2* by KBR.



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