AUGMENTATION OF TISSUE WATER PROTON SPIN-LATTICE RELAXATION RATES BY IN VIVO ADDITION OF PARAMAGNETIC IONS

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I. INTRODUCTION

Nuclear magnetic resonance (NMR) relaxation times of water protons in tissues have been studied for some years, with the goals of better understanding the interaction between water and other tissue components (1) and of contributing to medical diagnostic procedures (2). The possibility that naturally-occurring paramagnetic ions might contribute significantly to

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the observed relaxation rates has been raised, but has never been demonstrated (1). The potential for diagnostic applications of NMR relaxation times (or their reciprocals, the relaxation rates) has been enhanced by the development of zeugmatographic imaging techniques, which make possible the measurement of relaxation effects in intact macroscopic living organisms (3). The pronounced relaxation effects of very small concentrations of paramagnetic ions on water resonances in biological systems (4) have led to the suggestion that the deliberate introduction of such ions into living systems might give rise to interesting and useful changes in relaxation times (5,6).

II. MANGANESE IN VIVO

As a prelude to the use of relaxation contrast reagents in nuclear magnetic resonance zeugmatographic imaging, we have studied the effects of injections of manganous salt solutions on water proton spin-lattice relaxation times in the organs of dogs and rats. Manganese was chosen for the initial studies because manganous ion is a very effective water relaxation reagent (4), and because it is only moderately toxic. Also, the distribution of injected manganous ion in mammalian tissues, and its elimination, have been investigated (7,8). In preliminary experiments, to be reported elsewhere, it was shown that there was a significant enhancement factor for the relaxation of solvent water by manganous ion in rat plasma, that manganous ion concentrates *in vitro* in samples of myocardium, and that the distribution of stable manganese in the organs of rats and dogs, at dosages in the range 0.01 to 1 millimole/kg, resembled that of much smaller dosages of radioactive manganese isotopes (7).

III. MYOCARDIAL INFARCTION

In earlier work (9), it was shown that four hours after ligation of the canine anterior descending coronary artery, a region of significantly increased water proton spin-lattice relaxation time (T_1), and of increased water content, developed distal to the ligation. The effects were relatively small, with T_1 increases of about 10 to 20 per cent of the relaxation time of uninvolved myocardium, and there were greater differences between animals than between infarcted and normal myocardium in each heart. It seemed desirable, therefore, to investigate the possibility of differentially altering the

relaxation effects by the injection of a paramagnetic reagent that would preferentially affect either normal or infarcted, or possibly ischemic, myocardium. Our first choice, for the reasons noted above, was the manganous ion. An initial series of five dogs was studied, four with manganese injections and one control, with qualitatively consistent results. A summary of the data for one experiment, that on dog No. 4, will be presented here, and a complete account will be published elsewhere.

IV. EXPERIMENTAL

Adult mongrel dogs, 20-25 kg in weight, were fasted overnight and anesthetized with 30 mg/kg body weight of sodium pentobarbital. Inhibition of the corneal reflex was maintained with additional sodium pentobarbital as needed. The dogs were intubated and ventilated with a respirator, and 0.9 per cent normal saline was administered intravenously to replace fluid loss during surgery. The chest was opened through the fifth intercostal space, the lungs retracted, the pericardium opened, and the left anterior descending coronary artery dissected free and permanently occluded with a clamp. There was visible cyanosis distal to the occlusion on the anterior wall of the left ventricle.

After a 60 minute ischemic period, a saline solution containing manganous ion at a dosage of 0.1 mmole/kg body weight was injected into the left ventricle. Thirty minutes later, the heart was excised while still beating. The left ventricle was divided into a number of tissue blocks. The apparent spin-lattice relaxation time (T_1) was measured for each block by a $180°-\tau-90°$ pulse sequence at 4 MHz, and a least squares semi-exponential fit was used to obtain a single relaxation time. The water contents were measured by freeze-drying for 24 hours, and the manganese concentrations were measured by homogenizing the dried tissue, extracting with dilute nitric acid, and analyzing by atomic absorption spectrometry (10).

V. RESULTS

The results are given in Table I, and the locations of the samples are outlined on Figure 1. For comparison, the relaxation rates in normal and infarcted canine myocardium fall in the range 2.2 to 3.8 sec^{-1}, and the natural manganese concentration in the body, although rather variable, averages 3-6 micromoles/kg.

TABLE I. Manganese concentrations, water contents, and relaxation times and rates of samples of left ventricular myocardium of dog No. 4

Sample No.	T_1 (sec)	R_1 (sec^{-1})	Manganese concentration (mmol/kg)	Water content (per cent)
1	.221	4.52	.07	76.9
2	.237	4.22	.03	73.2
3	.263	3.79	.05	78.1
4	.167	5.97	.11	74.6
5	.144	6.94	.14	75.1
6	.284	3.52	.05	74.8
7	.314	3.19	.03	76.6
8	.336	2.98	.03	77.4
9	.269	3.71	.07	77.9
10	.193	5.18	.15	79.2
11	.138	7.27	.15	76.7
12	.142	7.04	.14	73.6
13	.321	3.12	.04	77.5
14	.274	3.65	.01	76.1
15	.451	2.22	.04	77.9
16	.166	6.04	.13	75.7
17	.101	9.25	.17	75.4
18	.121	8.24	.15	76.7
19	.224	4.46	.04	75.6
20	.205	4.88	.09	76.2
21	.239	4.19	.10	75.3
22	.234	4.27	.07	76.6
23	.089	11.24	.16	75.2
24	.137	7.27	.19	75.4
25	.166	6.02	.15	77.9
26	.124	8.06	.16	76.4
27	.151	6.62	.10	74.6
28	.107	9.38	.18	75.5
29	.128	7.82	.16	77.9
30	.118	8.44	.16	74.8
31	.142	7.06	.17	76.7
32	.146	6.86	.16	76.5
33	.136	7.36	.15	77.7
34	.086	11.63	.34	77.7
35	.114	8.78	.36	75.5
36	.111	9.03	.24	75.7
37	.117	8.56	.17	75.1
38	.112	8.93	.34	73.8
39	.117	8.57	.30	74.4
40	.097	10.34	.30	76.1

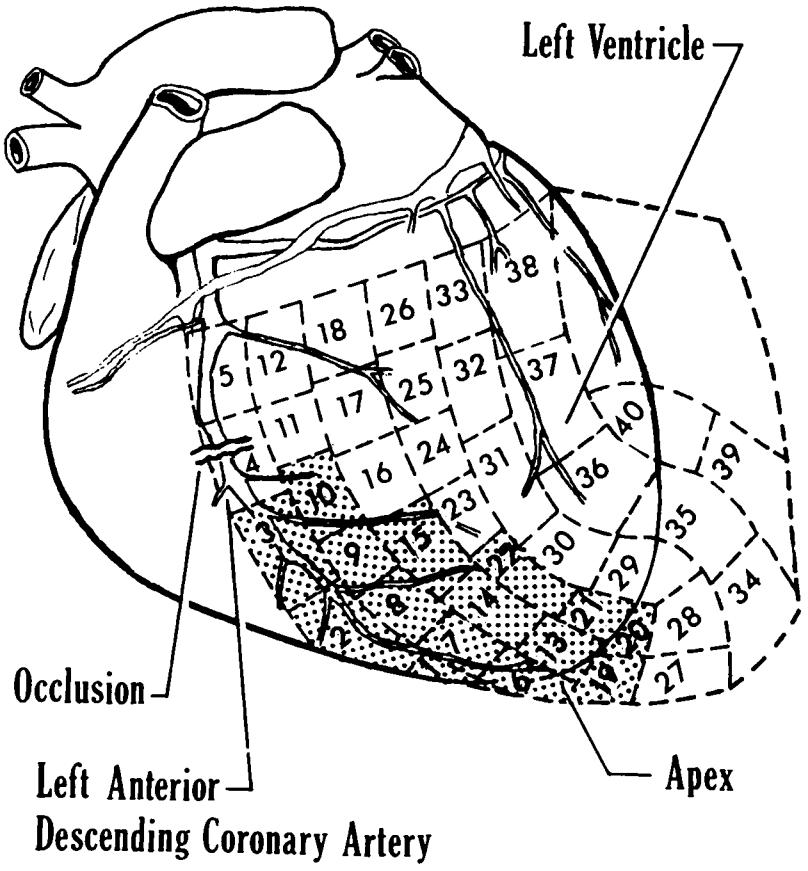

Fig. 1. The locations of samples for which data are given in Table 1. The region in which the relaxation rate R_1 is less than 6 sec^{-1} is stippled.

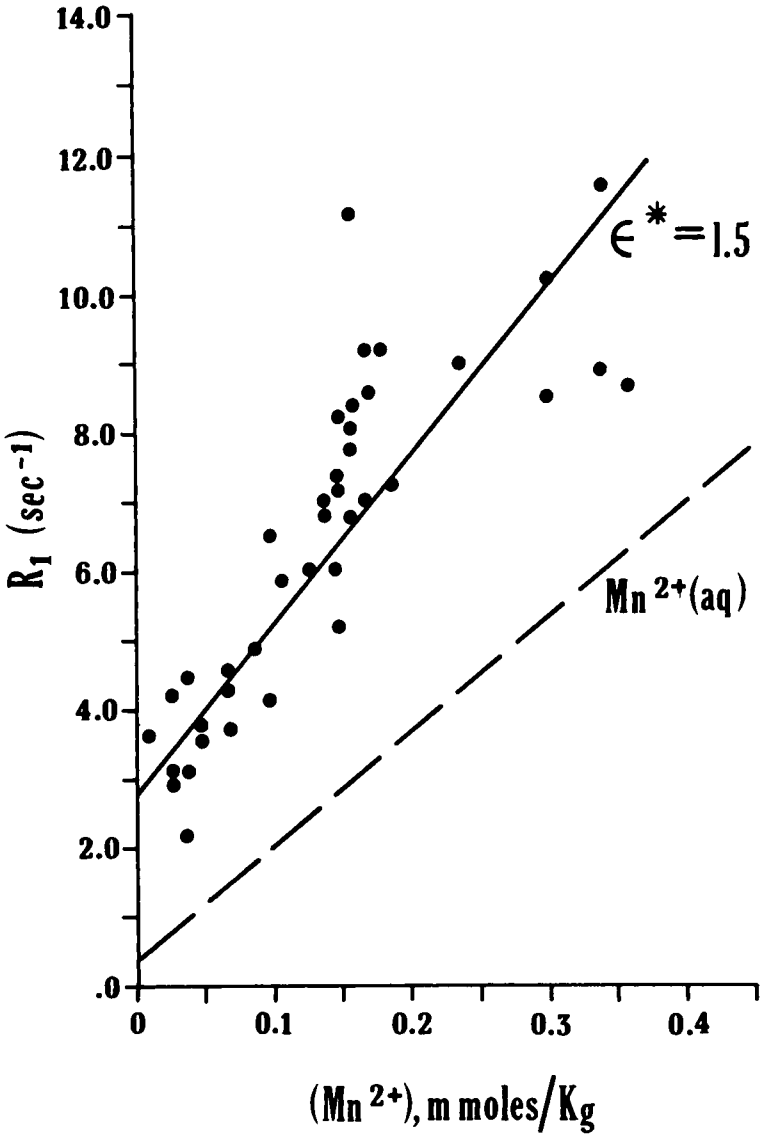

Fig. 2. The relationship between the relaxation rate (R_1) of the tissue water proton NMR signal at 4 MHz and the total manganese concentration in the tissue sample. The dashed line shows the same relationship for manganous ion in pure water, and the dotted line shows the concentration dependence to be expected if the manganese is present as manganous ion in the tissue, with an enhancement factor ϵ_1^*, of 1.5 and an intercept of 2.8 sec^{-1}.

VI. DISCUSSION

The ischemic region of the heart is clearly delineated by the relaxation rates and by the manganese concentrations, as shown in Figure 1. The ratios are similar to those recently observed in a study of the distribution of radioactive manganese in canine hearts when the isotope was injected three days after ligation of a coronary artery (11). Figure 2 shows the relationship between manganese concentration and relaxation rate in this one preparation. There are several factors that may account for the scatter of the points. Not only is each sample likely to be somewhat heterogeneous on a macroscopic scale, but there may also be intrinsic multicomponent relaxation behavior, similar to that observed for red blood cells in manganese-doped solutions (12). A tendency to cluster about a line defining an enhancement factor (4b) of 1.5 (assuming additivity of the intrinsic relaxation rate and that caused by manganous ion) is noticeable, however. In an NMR zeugmatographic imaging experiment, the T_1 contrast found in this work would permit the clear visualization of a region ischemic for only 90 minutes, although it is not clear whether the effects observed are the results of decreased perfusion or some other phenomenon.

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