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THE EFFECTS OF N-METHYL BUTYROHYDROXAMIC ACID AND OTHER MONOHYDROXAMATES ON REPERFUSION-INDUCED DAMAGE TO CONTRACTILE FUNCTION IN THE ISOLATED RAT HEART

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The effects of three novel methyl substituted monohydroxamates N-methyl butyrohydroxamic acid (NMBH), N-methyl acetohydroxamic acid (NMAH) and N-methyl benzohydroxamic acid (NMBzH) against reperfusion induced contractile dysfunction were investigated in the isolated Langendorff-perfused rat heart. All these drugs produced an improved recovery of left ventricular developed pressure (LVDP) compared to control hearts in the order NMBH* (65 ± 8%) > NMAH (59 ± 8%) > NMBzH (48 ± 3%) > control (35 ± 3%) (mean ± s.e.), however only the recovery obtained in NMBH treated hearts was significantly different from control hearts (*$p > 0.05$, Dunnett's test). Both NMAH (98 ± 10%) and NMBH (84% ± 8) produced a significant improvement in the recovery of heart rate (control 48 ± 13%). There was no significant improvement of coronary flow, and NMBzH-treated hearts showed a significant reduction in recovery. The improved recovery in both LVDP and heart rate obtained with NMBH suggests this drug may be effective in attenuating reperfusion-induced contractile dysfunction in the isolated rat heart. Further, a comparison of the structures of the hydroxamates described in this study with the results obtained with desferrioxamine, (a trihydroxamate), and N-methyl hexanoylhydroxamic acid (NMHH) in other studies suggests that the nature of the alkyl chain attached to the carbonyl group of the hydroxamate may contribute to the efficacy of monohydroxamates in attenuating this type of myocardial injury in this model.

KEY WORDS: heart, desferrioxamine, hydroxamates, ischaemia-reperfusion injury, free radicals.

INTRODUCTION

Experiments have a suggested a link between myocardial dysfunction on reperfusion after a period of ischaemia and the generation of free radicals.[1-7] The trihydroxamate desferrioxamine has been shown both to scavenge free radicals[8,9] such as the hydroxyl, ferryl myoglobin and superoxide radicals as well as to chelate iron, one of the catalytic components in the generation of initiating species.[7,10,11] As a result of these properties its ability to ameliorate some aspects of reperfusion injury has been tested.[4,10,12-15] However, the effectiveness of desferrioxamine treatment is controversial and the degree of success varies with different models and perfusion

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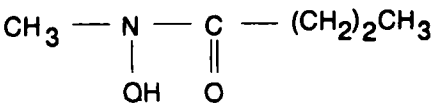
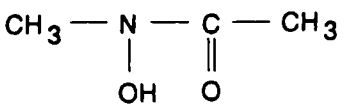


N-methyl butyrohydroxamic acid (NMBH)



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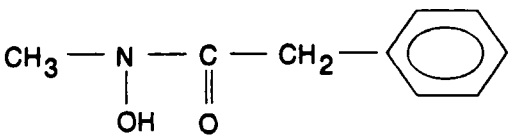

N-methyl benzohydroxamic acid (NMBzH)

FIGURE 1 The structures of N-methyl butyrohydroxamic acid (NMBH), N-methyl acetohydroxamic acid (NMAH) and N-methyl benzohydroxamic acid (NMBzH).

protocols.[16-19] Free radicals have been shown to appear within the first minutes of reperfusion[13-15] and it has been suggested that desferrioxamine principally enters those cells actively undergoing pinocytosis[20,21] and therefore does not necessarily reach the sites of intracellular free radical generation on a physiologically-relevant time scale, which may limit its potential therapeutic usefulness.

Little is known of the biochemical or physiological properties of other compounds containing the hydroxamate group[22] and to investigate the potential of these substances further, we have synthesised a series of methyl-substituted mono-hydroxamic acids. In previous studies, we have examined some of their biochemical properties[23,24] and compared the effects of one of them, N-methyl hexanoyl-hydroxamic acid (NMHH), with the trihydroxamate desferrioxamine and the thiol compound N-acetylcysteine against reperfusion-induced dysfunction in the rat heart.[12] In the current work, we have studied the effects of three novel methyl-substituted derivatives: N-methyl butyrohydroxamic acid (NMBH), N-methyl acetohydroxamic acid (NMAH) and N-methyl benzohydroxamic acid (NMBzH) (Figure 1) on reperfusion-induced myocardial contractile dysfunction in the isolated rat heart with a view to investigating the therapeutic potential of hydroxamate-containing compounds and the effect of differing substituents on their efficacies.

MATERIALS AND METHODS

Adult male Wistar rats weighing between 240–320 g were anaesthetised i.p. with sodium pentobarbitone (Saggital; 60 mg/kg bwt) and heparinsed (100 IU/100 g bwt) via the femoral vein. The heart was excised into cold Krebs and mounted for perfusion using the Langendorff technique at constant pressure (100 cm H_20). The

hearts were perfused with Krebs bicarbonate buffer consisting of NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.6, $MgSO_4$ 1.2, KH_2PO_4 0.8, Glucose 7.6, $CaCl_2$ 1.4 (all mM; supplied by BDH). The buffer was gassed with 95% O_2/5% CO_2 and the solution and apparatus was maintained at 37°C.

A ballon was inserted into the left ventricle and inflated to a left ventricular end diastolic pressure (LVEDP) of 4 mmHg. The balloon remained inflated throughout the experiment and was connected to a chart recorder via a pressure transducer. The balloon was used to measure left ventricular peak systolic pressure (LVPSP), LVEDP, left ventricular developed pressure (LVDP, =LVPSP−LVEDP) and heart rate. In addition, the coronary effluent was collected over timed intervals as a measure of the coronary flow. These assessments of function were made 20 min after the start of aerobic perfusion and immediately before ischaemia (30 min). These parameters were reassessed at 10 min intervals, commencing 30 min after the start of reperfusion and continuing until the end of the experiment. Percentage recovery was calculated from the values obtained at the end of 60 min reperfusion expressed as a percentage of the values obtained immediately before the ischaemic insult. Global (whole heart) ischaemia (non-perfusion) was produced by clamping the perfusion flow into the aorta. The coronary effluent was collected during the first 10 min of reperfusion and analysed for creatine kinase leakage (Randox Laboratories analytical kit). The experiments were completely randomised. Data is expressed as mean ± s.e., comparison of drug-treated hearts with control hearts was by Dunnett's test,[25] p values <0.05 were considered significant.

Perfusion Protocols

Preliminary experiments using different periods of ischaemia and reperfusion were carried out to determine the most suitable ischaemic and reperfusion times. These pilot studies showed that 50 min ischaemia followed by 60 min reperfusion produced the desired 30–50% recovery of LVDP and a stable heart rate. Therefore the hearts were subjected to 30 min aerobic perfusion, followed by 50 min global ischaemia and 60 min reperfusion (Figure 2). Drug treated hearts followed a similar protocol except that the appropriate drug (all at 150 μM) was present in the perfusate for the first 10 min of reperfusion following the ischaemic insult.

The drugs used were produced by standard laboratory methods.[26]

RESULTS

Control Hearts

The absolute preischaemic haemodynamic values of the control hearts were;- LVDP 90 ± 5 mmHg, heart rate 281 ± 9 bpm, and coronary flow 12 ± 1 ml/min. Using one way analysis of variance, there was no significant difference between these preischaemic values and those of any of the drug-treated hearts, showing that they came from a homogenous population. Ischaemia initially produced a cessation of the normal heart beat and a loss of ventricular tone, followed by a slow constricture of the myocardium which reached a peak in approximately 20–30 min, and subsequently decreased slightly. Reperfusion produced an additional rapid constricture and an arrhythmogenic heart beat. The constricture slowly declined with further reperfusion and LVDP was 20±6% of the preischaemic value at 30 min when

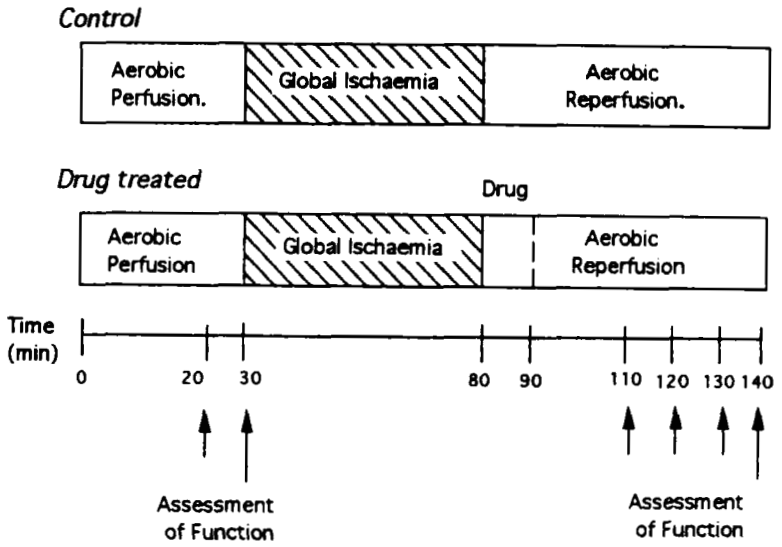

FIGURE 2 The perfusion protocols.

haemodynamic function was reassessed, and improved to a final recovery of 35 ± 8% after 60 min reperfusion (Figure 3A).

Drug-treated hearts

Drug-treated hearts tended to show an improved recovery of contractile function compared to controls as indicated by LVDP (Figure 3A). The largest improvement being with NMBH > NMAH > NMBzH > control. However, NMBH was the only one of the three drugs where recovery of LVDP was significantly different from that achieved by the control. Similarly, all the drug-treated hearts tended to show an improved recovery of heart rate compared to control (Figure 3B). The order of improvement was NMAH > NMBH > NMBzH with both NMAH and NMBH showing recoveries that were statistically significantly different from control ($p < 0.05$). There was no improvement in coronary flow with the drug treated hearts (Figure 3C), and with NMBzH there was a significant reduction in the final recovery of coronary flow. Further, there was no significant difference in the creatine kinase leakage for the first 10 min of reperfusion after ischaemia between the control and the drug-treated hearts (Figure 4).

DISCUSSION

There is increasing evidence that oxygen-derived radicals are produced in the myocardium on reperfusion after an ischaemic episode.[2,3,5-8,27] The reduction of oxygen to superoxide is the first stage in a series of reactions which is capable of generating in vitro the hydroxyl and ferryl haem protein species.[5,27,28] Superoxide itself is not very reactive per se[28] and would not be expected to account for the cell damage observed in ischaemia-reperfusion injury. The more reactive hydroxyl

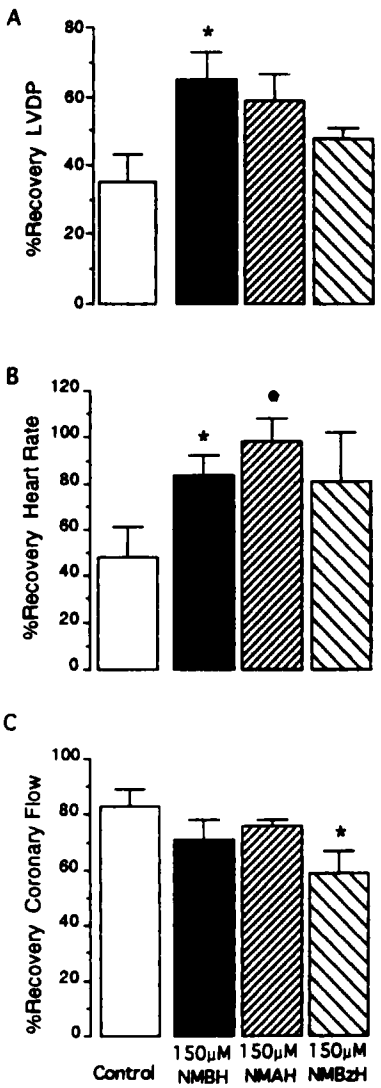

FIGURE 3 The effects of NMBH, NMAH and NMBzH (all at 150 μM) present in the perfusate for the first 10 min of reperfusion after ischaemia on the recovery of A. LVDP; B. Heart Rate; C. Coronary Flow. (Mean ± s.e shown; n = 10 control and n = 8 all drug-treated groups).

radical as well as ferryl haem protein species, both formed from hydrogen peroxide produced by superoxide dismutation, have been implicated as active mediators of cell damage in ischaemia-reperfusion injury.[5,6,27] One of the proposed mechanisms for hydroxyl radical reactions is the cycling of redox-active iron ions released from sources such as transferrin,[30] ferritin,[30] myoglobin[9,31] and haemoglobin[32] as a result of oxidative stress.

There has been considerable interest in substances capable of intervening in these reactions and attenuating the damaging effects of reperfusion. One such substance

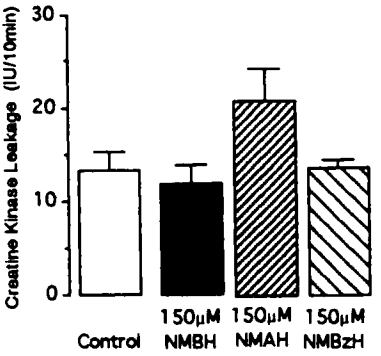

FIGURE 4 The effects of NMBH, NMAH and NMBzH (all at 150 μM) in the perfusate for the first 10 min of reperfusion after ischaemia on creatine kinase leakage during this reperfusion period. (Mean ± s.e shown; n = 10 control and n = 8 all drug-treated groups).

is the trihydroxamate desferrioxamine, which has been shown to both chelate iron, hence inhibiting redox cycling and radical generating reactions,[7,10,11] and to have radical scavenging properties of its own.[8,9] Apart from our previous study with NMHH in the isolated rat heart,[12] desferrioxamine is the only hydroxamate compound which has been previously investigated for its ability to attenuate myocardial dysfunction following ischaemia and reperfusion. However, the successful use of this compound is limited. It has been shown that if desferrioxamine is given *before* reperfusion it can provide *acute* protection of myocardial function,[15,19] however, it does not provide protection if it is given *on* or *after* reperfusion[10,12] neither does it provide *chronic* protection, with the treatment regimes so far described.[19]

The possible reasons for limited protection may be related to the pharmaco-kinetic properties of this drug. In particular it principally enters only those cells undergoing active pinocytosis,[20,21] has a relatively short half-life of 5–7 minutes in the plasma[33] and can exhibit pro-oxidant effects at high concentrations.[34] The selective penetration of the drug into cells could account for the requirement to administer the drug before reperfusion and its subsequent lack of effect when applied on reperfusion. Furthermore, although desferrioxamine might attenuate an initial burst of free radical production, because of its low permeability and short half-life, it would be expected to have little effect on the production of free radicals in cells not undergoing pinocytosis or on the chronic production of free radicals, e.g. by activated neutrophils[35] once treatment was discontinued. However, despite this evidence that desferrioxamine offers acute rather than chronic protection,short term attenuation of myocardial dysfunction on reperfusion is still an essential goal since it results in the continued functioning and possible ultimate viability of hearts which would otherwise fail at an early stage.

The novel hydroxamic acids described in this study have been reported to possess radical scavenging activity since they are capable of reducing ferryl myoglobin to metmyoglobin.[24] The effectiveness of these compounds in attenuating reperfusion-induced contractile injury is however unlikely to depend solely on this property. Their relative rates of reaction in terms of their suppressive effects on the activation of ferryl myoglobin are NMHH > NMBzH > NMAH > DFO > NMBH.[24] This is a

different order to that of the percentage recovery of LVDP reported in this and our other study[12] i.e. NMBH > NMHH > NAMH > DFO > NMBzH > control. The increased efficacies of some of these monohydroxamate drugs over the trihydroxamate desferrioxamine suggest that the efficacy of these drugs is independent of the *number* of hydroxamate groups. Their size and the uncharged nature of their structure compared to that of desferrioxamine might be expected to result in an enhanced rate of cellular penetration. This is of particular relevance in non-blood perfused models where the absence of activated neutrophils means that free radical generation is more likely to occur intracellularly as a result of for example, aberrantly-functioning mitochondria[37,38] and endothelium.[37,39] Furthermore, consideration of the structures of NMBH and NMHH indicates that the nature of the alkyl chains attached to the carbonyl groups might be a factor affecting the relative efficacies of these drugs against reperfusion-induced contractile damage.

The principal other antioxidant and free radical scavenging therapeutic interventions investigated to date include: (i) thiol-containing amino acid derivatives such as N-acetyl cysteine (NAC) which are able to supplement the normal glutathione defence system or supply the appropriate reducing equivalents,[40] and have radical scavenging properties of their own,[8] and (ii) superoxide dismutase (SOD) which converts superoxide radicals to hydrogen peroxide and is often used in conjunction with catalase which then reacts with hydrogen peroxide to form water and oxygen.[41] However, the efficacies of all of these interventions are controversial. In general while the use of all these agents prior to or during ischaemia has been shown in a number of investigations to limit reperfusion dysfunction;[10,12,15,40,42-44] their application after ischaemia (i.e. on reperfusion) has been less successful and their application may only delay necrosis.[17,19,42,43,45,46]

CONCLUSIONS

The results of this study are consistent with the proposition that the admistration of iron chelators and free radical scavengers is effective in the attenuation of acute reperfusion-induced contractile dysfunction after ischaemia. The literature shows however that there are limitations to the usefulness of present potential interventions which could be related to the pharmaco-kinetic properties of the compounds used and the treatment regimes. As the nature of free radical-induced myocardial injury is more fully understood the effectiveness of potential interventions will improve. One of the novel methyl monohydroxamates described in this study, NMBH, appears to have more favourable pharmaco-kinetic properties than the other known hydroxamates and is effective in attentuating reperfusion induced contractile injury in the isolated rat heart.

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