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Beneficial effects of N-acetylcysteine and cysteine in stunned myocardium in perfused rat heart

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1 The objective of this study was to evaluate the effects of three sulphydryl (SH) compounds, N-acetylcysteine (NAC), cysteine (Cys) and cystamine, on functional recovery and ventricular arrhythmias (VF) in stunned myocardium in the isolated perfused heart of the rat.

2 Hearts ($n = 7$–8 per group) were perfused by the Langendorff procedure for 20 min to stabilize and then assigned to one of five groups: saline, sham, NAC, Cys and cystamine. After the stabilizing period, the drugs (at 3.6 μM min^{-1}) or their vehicle (saline) were infused into coronary vessels throughout the experimental period. Ten min after administration of drugs, the left anterior descending coronary artery (LAD) was ligatured for 20 min and then untied to reperfuse for 30 min. In the sham group, a ligature was placed around the LAD but not tied.

3 NAC and Cys had a significant effect in attenuating myocardial stunning: the percentage recovery of rate-pressure product measured 30 min after reperfusion as an index of heart function, was improved with the NAC (98.3 ± 4.5) and Cys groups (104.0 ± 6.5) compared with the saline (only 73.6 ± 3.8, $P < 0.01$) group. Cystamine did not show these beneficial effects. This may be due to the difference in chemical structure between NAC, Cys and cystamine since the latter does not have a free SH group with a disulphide bond formed. This phenomenon suggests that a free SH group is essential for the protective effects of compounds like NAC and Cys in myocardial injury.

4 NAC and Cys prevented the fall in coronary flow during the LAD occlusion and enhanced coronary flow during reperfusion but cystamine did not have such a beneficial effect.

5 The incidence of VF in the saline, cystamine, Cys and NAC groups was 6/8 (75.0%), 4/7 (57.1%), 3/8 (37.5%) and 2/7 (28.6%), respectively, and no significant differences ($P > 0.05$) were noted between the saline- and drug-treated groups.

6 An *in vitro* study with electron spin resonance indicated that Cys effectively scavenged the hydroxyl radical (·OH) generated by Fenton's reaction but did not scavenge superoxide generated in an irradiated riboflavin system. NAC and cystamine showed a scavenging effect on ·OH to a certain extent but this effect did not reach statistical significance ($P > 0.05$ vs saline).

7 Our results demonstrate that NAC and Cys treatment before ischaemia and reperfusion can reduce myocardial stunning. This beneficial effect may be mainly due to their ability to preserve and enhance coronary flow during coronary occlusion and reperfusion and in part due to scavenging ·OH and/or replenishing intracellular glutathione. The results also indicate that the condition of coronary perfusion can produce a great impact on postischaemic ventricular performance.

Introduction

Re-establishment of the blood flow to ischaemic myocardium is essential for tissue survival and functional recovery. However, many studies have demonstrated that reperfusion also brings about some deleterious effects, i.e., reperfusion injury (Braunwald & Kloner, 1985; Becker & Ambrosio, 1987; Opie, 1989). Recent evidence suggests that postischaemic myocardial dysfunction (stunning) may represent a functional or nonlethal form of reperfusion injury (Braunwald & Kloner, 1982; Kloner *et al.*, 1989) and oxygen free radicals may contribute to the pathogenesis of myocardial stunning (Gross *et al.*, 1986; Bolli *et al.*, 1987; 1989; Forman *et al.*, 1988; Faber *et al.*, 1988). Bolli and his colleagues (1988) using electron paramagnetic resonance spectroscopy detected a burst of free radical formation within the coronary venous effluent in dogs subjected to 15 min of coronary occlusion and reperfusion; other studies found that production of free radicals increased markedly in isolated hearts of rabbit or rat undergoing global ischaemia and reperfusion (Zweier *et al.*, 1987; Garlick *et al.*, 1987; Kramer *et al.*, 1987) and pretreatment with the oxygen radical-scavenging agents superoxide dismutase and catalase reduced the degree of left ventricular dysfunction in canine models of stunned myocardium (Gross *et al.*, 1986; Ambrosio *et al.*, 1987; Przyklenk & Kloner, 1989). Nevertheless, recent studies also indicate that other factors independent of free radicals are involved in the pathogenesis of myocardial stunning such as: calcium overload (Kusuoka *et al.*, 1987; Lee *et al.*, 1987; Steenbergen *et al.*, 1990; Sun & Lin, 1990), heterogeneous impairment of myocardial perfusion (Stahl *et al.*, 1986) and neutrophil accumulation in the postischaemic myocardium (Westlin & Mullane, 1989) and so on.

NAC and Cys are sulphydryl (SH) compounds which have been used recently as antioxidant agents in myocardial ischaemia and reperfusion and have shown some beneficial effects (Forman *et al.*, 1988; Ceconi *et al.*, 1988; Tang & Tang, 1991). Previously, SH compounds have been shown to have anti-inflammatory properties and can protect against irradiation and drug-induced disease states in which free radicals have been implicated as mediators of tissue injury (Suguhara *et al.*, 1977; Doroshow *et al.*, 1980; Forman *et al.*, 1983; Betts *et al.*, 1984) through such effects as: reacting directly with free radicals, promoting the resynthesis of glutathione (GSH) or

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acting as an alternative substrate for glutathione peroxidase. It has also been shown that NAC potentiates both the peripheral and coronary vasodilator effects of nitroglycerin (Horowitz et al., 1983; Winniford et al., 1986). The latter findings suggest that NAC may work through mechanisms other than as an oxidant scavenger to benefit the ischaemic myocardium.

The purpose of the present study is to examine whether the SH compounds: NAC, Cys and cystamine have therapeutic actions in a rat heart model of brief ischaemia. If these compounds have these actions, how do they produce the effects, as free radical scavengers or via other actions? In order to understand better the mechanisms by which the drugs in this study might be capable of acting as oxidant scavengers *in vivo*, we used the electron spin resonance (ESR) method to evaluate the scavenging effects of NAC, Cys and cystamine on superoxide ($\cdot\bar{O}_2$) and hydroxyl radicals ($\cdot$OH) *in vitro*. Furthermore, since cystamine does not have a free SH group for a formed disulphide link, we could also determine whether the free SH group is necessary for the prevention of ischaemia and reperfusion injury.

Methods

Heart perfusion

Wistar rats of either sex weighing between 250–350 g were given 1000 iu kg^{-1} of heparin intraperitoneally (i.p.) 15 min prior to the induction of anaesthesia with sodium pentobarbitone (40 mg kg^{-1}, i.p.). Following a thoracotomy, the hearts were rapidly excised and placed in ice-cold oxygenated (95% O_2:5% CO_2) Krebs-Henseleit (K-H) solution which contained (in mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.1, $CaCl_2 \cdot 2H_2O$ 2.5, $NaHCO_3$ 25 and glucose 11. Within 30 s, the hearts were perfused retrogradely via the aorta with oxygenated K-H solution according to the Langendorff technique as described earlier (Sun & Lin, 1990) and by Roth et al. (1985). The ventricular pressure was monitored by placing a plastic catheter with a small perforated ball tip into the left ventricle via the mitral valve. The catheter was filled with perfusate and connected to a Statham P50 pressure transducer. Coronary perfusion pressure (CPP) was measured in a side branch of the inflow line with a Statham P50 pressure transducer. Left ventricular pressure, left ventricular *dp/dt* and (−) *dp/dt* and CPP were recorded on a polygraph recorder (RM-46, Nihon Kohden). The rate-pressure product (RPP) was determined by calculating heart rate times left ventricular developed pressure (LVDP, systolic minus diastolic pressure). The coronary flow rate (CFR) was measured from collection of the coronary effluent every minute.

Protocol

At all times, the hearts were maintained at 37°C and perfused with normal K-H buffer at a constant pressure of 60 mmHg. After a 25 min equilibration period, hearts were assigned to one of five groups: NAC, Cys, cystamine, saline and sham. The baseline recordings were taken at the end of the equilibration period (designated time 0) and ischaemia was produced by tying a previously placed reversible ligature around the left anterior descending coronary artery (LAD) at a point close to its origin. Infusion of NAC, Cys and cystamine (each at 18 mM) or their vehicle (0.9% NaCl), via a peristalic pump (model HL-2, Shanghai) into the aortic inflow cannula at a rate of 0.2 ml min^{-1}, corresponding to a final concentration of 3.6 μM min^{-1}, was started 10 min before coronary artery occlusion and continued for the duration of the experiment. The concentrations of drugs chosen were based on data obtained in our previous experiments in which NAC and Cys at the above dose showed a significant effect in reducing reperfusion-induced arrhythmias in the transient ischaemic model of rat heart (Tang & Tang, 1991) and also is comparable with the concentration used by Ceconi et al. (1988). In a sham group, all procedures were the same as the other groups except that the ligature around the LAD was not tied and no drug was infused.

Assays of scavenging effects of the drugs

The ESR spin trapping methods were used to assess the ability of the drugs to react with $\cdot\bar{O}_2$ and $\cdot$OH which were produced by the following methods. The assays for each drug were performed at least three times.

The irradiated riboflavin reaction: the reaction solution contained: 3×10^{-4} M riboflavin, 0.1 M 5,5-dimethyl-1-pyrroline N-oxide (DMPO, Sigma Chemical, St. Louis, Missouri, U.S.A.), 5×10^{3} M ethylenedinitrolotetraacetic acid (EDTA), 3.6×10^{-4} M of the drug dissolved in saline (these concentrations of drugs were equivalent to the concentrations of drugs which were infused into the coronary vessels in this experiment) and 0.05 M Na_2HPO_4–NaH_2PO_4 buffer at pH 5.0; this solution was mixed evenly and then a sample from the mixture was placed in an optical cavity (ER 4104 OR), irradiated with a Xenon lamp (150 W, distance: 30 cm) for 10 s and immediately thereafter ESR spectra were recorded with the ESR spectrograph (Bruke ESP-300, F.R.G.) under the following conditions: microwave power: 10 mW, modulation frequency and amplitude: 100 kHz and 0.2 G, time constant: 20 ms, at 25°C.

Fenton's reaction: the reaction mixture contained: 1.25% H_2O_2, 2×10^{-4} M $(NH_4)_2FeSO_4$, 0.1 M DMPO, 3.6×10^{-4} M drug dissolved in saline, 0.05 M Na_2HPO_4-NaH_2PO_4 buffer at pH 7.4; this was shaken quickly for 4 min. A sample from the mixture was put into a TE102 cavity (ER 4102 ST) and then ESR spectra were recorded as above, except that modulation frequency and amplitude were 25 kHz and 1.0 G, respectively.

Statistics

The results are presented as mean ± s.e.mean. Differences between experimental groups were analysed by one-way analysis of variance and when this test indicated statistical significance, the results from different groups were analysed by Student's nonpaired *t* test for multiple comparison to the cystamine or saline group. The incidence of ventricular fibrillation (VF) was analysed by Fisher's exact test. The probability was considered significant if less than 0.05.

Chemicals

Three compounds: NAC, Cys and cystamine were provided by the Pharmaceutical Laboratory in our institute. Their chemical structures are illustrated in Figure 1.

Results

Changes of heart function parameters

In the period of reperfusion after exposure to 20 min of LAD occlusion, ventricular function was severely depressed in saline and cystamine groups; however, the recovery of ventricular function indicated by the percentage recovery of RPP was significantly better in the cys and NAC groups (Figure 2). LVDP and (±)*dp/dt* also showed improvement to a varying extent in the Cys and NAC groups as compared with saline and cystamine groups (Table 1). Heart rate (HR) appeared to be

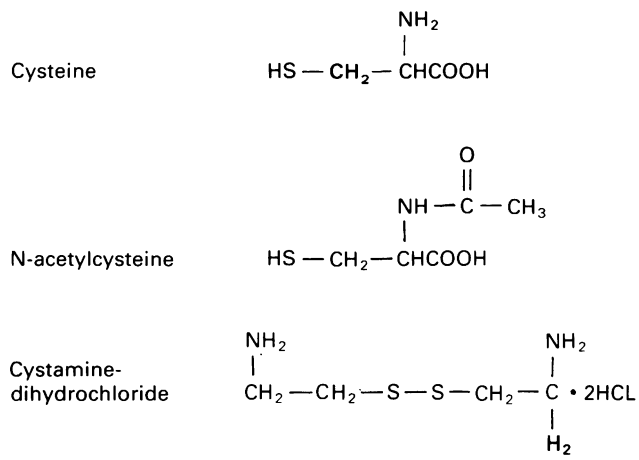

Figure 1 Structure of the drugs used in the experiments.

decreased during occlusion and reperfusion in the saline and cystamine groups though significant differences were not noted between groups ($P > 0.05$, Table 1). Obviously, both LVDP and HR contributed to changes in RPP; however, it was mainly drug-induced changes in LVDP that underlay the change in RPP.

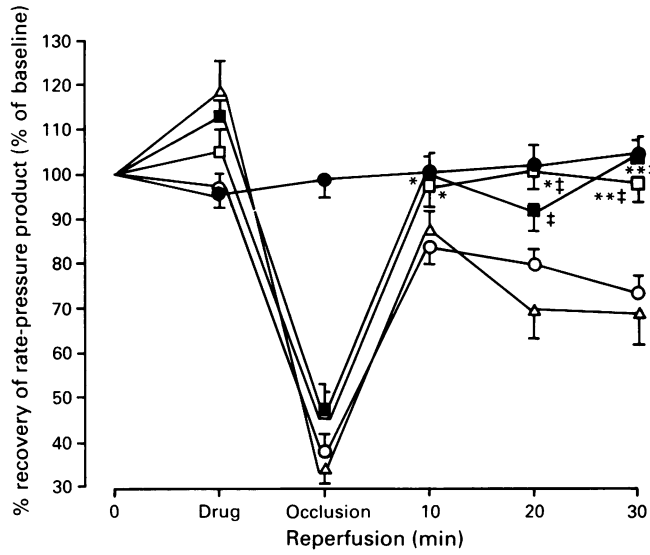

Figure 2 Rate-pressure product expressed as a percentage of the baseline value. Drug: 10 min after infusion of saline or drugs. Occlusion: 20 min after the LAD occlusion. (●) Sham; (■) cysteine; (□) N-acetylcysteine; (○) saline; (△) cystamine. Each point is mean with s.e.mean shown by vertical bars. * $P < 0.05$; ** $P < 0.01$ vs saline; † $P < 0.05$; ‡ $P < 0.01$ vs cystamine.

Table 1 Effects of N-acetylcysteine (NAC), cysteine (Cys) and cystamine on left ventricular function in stunned myocardium in perfused rat heart

	HR (beats min^{-1})	*LVDP* (mmHg)	dp.dt^{-1} (mmHg s^{-1} × 10^{-3})	(−)dp.dt^{-1} (mmHg s^{-1} × 10^{-3})	*CFR* (ml min^{-1})
0 (Baseline)					
Saline	248 ± 18	67 ± 1	1.55 ± 0.08	1.08 ± 0.05	9.1 ± 0.6
Sham	235 ± 14	64 ± 3	1.47 ± 0.07	1.20 ± 0.07	8.9 ± 0.6
NAC	232 ± 16	69 ± 2	1.50 ± 0.09	1.06 ± 0.08	9.1 ± 0.6
Cys	253 ± 27	61 ± 3	1.36 ± 0.08	0.92 ± 0.09	10.2 ± 0.9
Cystamine	223 ± 13	70 ± 7	1.52 ± 0.12	1.13 ± 0.11	8.7 ± 0.5
10 min after drugs infusion					
Saline	239 ± 17	67 ± 2	1.56 ± 0.08	1.10 ± 0.06	9.3 ± 0.6
Sham	226 ± 11	63 ± 3	1.49 ± 0.06	0.97 ± 0.06	8.9 ± 0.7
NAC	235 ± 19	72 ± 4	1.61 ± 0.15	1.12 ± 0.11	10.6 ± 0.9
Cys	256 ± 25	69 ± 5	1.61 ± 0.11	1.07 ± 0.11	11.3 ± 1.0
Cystamine	253 ± 11	73 ± 8	1.69 ± 0.18	1.26 ± 0.13	13.0 ± 1.3*
20 min after occlusion					
Saline	209 ± 16	30 ± 3	0.64 ± 0.06	0.36 ± 0.06	6.6 ± 0.5
Sham	239 ± 10	64 ± 3	1.48 ± 0.06	1.02 ± 0.06	9.5 ± 0.9**
NAC	222 ± 13	34 ± 5	0.76 ± 0.07†	0.44 ± 0.06	8.9 ± 0.5**
Cys	229 ± 29	33 ± 5	0.72 ± 0.07	0.42 ± 0.04	9.1 ± 0.7**
Cystamine	220 ± 12	24 ± 3	0.51 ± 0.07	0.31 ± 0.04	8.5 ± 0.9
10 min after reperfusion					
Saline	242 ± 20	59 ± 3	1.32 ± 0.05	0.86 ± 0.05	10.3 ± 0.6
Sham	243 ± 11	61 ± 4	1.41 ± 0.06	1.01 ± 0.05	9.4 ± 0.7
NAC	244 ± 15	66 ± 5	1.51 ± 0.13†	1.06 ± 0.10	13.1 ± 1.1*
Cys	234 ± 30	63 ± 3	1.39 ± 0.08	0.94 ± 0.06	13.1 ± 1.3
Cystamine	240 ± 15	44 ± 10	1.10 ± 0.12	0.77 ± 0.08	11.5 ± 0.9
20 min after reperfusion					
Saline	231 ± 22	58 ± 4	1.36 ± 0.06	0.88 ± 0.06	9.6 ± 0.8
Sham	246 ± 11	61 ± 4	1.50 ± 0.06	1.01 ± 0.07	9.5 ± 0.6
NAC	237 ± 17	70 ± 4*†	1.58 ± 0.13‡	1.09 ± 0.10†	12.6 ± 1.1*
Cys	237 ± 28	60 ± 3	1.35 ± 0.07†	0.88 ± 0.09	12.5 ± 1.2
Cystamine	226 ± 16	45 ± 8	0.92 ± 0.16*	0.66 ± 0.12	10.7 ± 0.8
30 min after reperfusion					
Saline	212 ± 23	58 ± 4	1.28 ± 0.07	0.78 ± 0.06	8.7 ± 0.6
Sham	257 ± 16	61 ± 3	1.54 ± 0.03	1.06 ± 0.08	9.6 ± 0.9
NAC	229 ± 17	70 ± 4†	1.59 ± 0.13†	1.08 ± 0.10*†	12.0 ± 1.1*
Cys	230 ± 30	62 ± 4	1.33 ± 0.09	0.88 ± 0.09	11.8 ± 1.1*
Cystamine	206 ± 17	54 ± 4	0.93 ± 0.17	0.61 ± 0.14	9.8 ± 1.0

$n = 7$–8 in each group.
HR, heart rate, LVDP, left ventricular developed pressure, i.e., the difference between systolic and end-diastolic pressure, *dp/dt* and (−)*dp/dt*, rate of left ventricular pressure rising and falling, CFR, coronary flow rate. * $P < 0.05$, ** $P < 0.01$ vs saline; † $P < 0.05$, ‡ $P < 0.01$ vs cystamine.

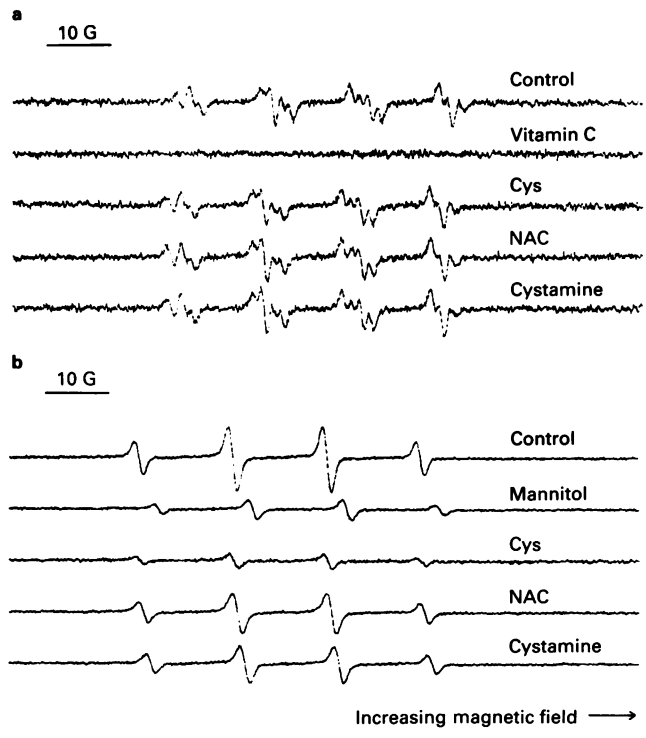

Figure 3 (a) ESR spectra of $\cdot\bar{O}_2$ generated from aqueous solution system by spin trapping of 5,5-dimethyl-1-pyrroline N-oxide (DMPO). Control: 3×10^{-4} M riboflavin, 0.1 M DMPO, 5×10^{-3} M EDTA, 0.05 M PBS, pH = 5.0. Cysteine (Cys), N-acetylcysteine (NAC), cystamine and vitamin C: the conditions were the same as control except that Cys, NAC, cystamine or vitamin C were added. Cys, NAC and cystamine as in Table 1. (b) ESR spectra of ·OH generated from aqueous solution by spin trapping of DMPO. Control: 1.25% H_2O_2, 2×10^{-4} M $(NH_4)_2$ $FeSO_4$, 0.1 M DMPO, 0.05 M PBS, pH = 7.4. Mannitol, cysteine (Cys), N-acetylcysteine (NAC) and cystamine: the conditions were the same as control except that mannitol, Cys, NAC and cystamine were added respectively. In this study, vitamin C and mannitol were used as a positive control for scavenging $\cdot O_2$ and ·OH, respectively. In this figure, centre field and sweep width are 3420.00 and 100.0 Gauss, respectively.

The haemodynamic profiles of the five groups, suggested that NAC and Cys had beneficial effects on recovery of heart function.

Changes of coronary flow rate (CFR)

After 10 min of cystamine administration, CFR increased significantly ($P < 0.05$ vs saline). This indicated that cystamine could enhance CFR in the perfused rat heart under non-ischaemic conditions. During the LAD occlusion period, CFR decreased in the saline group but this fall was prevented in the NAC and Cys groups ($P < 0.05$ vs saline); during the 30 min reperfusion period, CFR increased significantly in the NAC and Cys groups ($P < 0.05$ vs saline), indicating that NAC and Cys can improve coronary flow during both coronary occlusion and reperfusion periods. Cystamine did not show the beneficial effect on CFR throughout the occlusion and reperfusion periods (Table 1).

Effect of the drugs on arrhythmias

In this experiment, the incidence of ventricular fibrillation (VF) after reperfusion was compared between groups. There was no VF in the sham group during the reperfusion period. The incidence of VF after reperfusion in the saline, cystamine Cys and NAC groups was 6/8 (75.0%), 4/7 (57.1%), 3/8 (37.5%) and 2/7 (28.6%), respectively and no significant differences ($P > 0.05$) were noted between the NAC, Cys, cystamine and saline groups though NAC and Cys showed a strong tendency to reduce the incidence of VF in this study.

Scavenging effects of the drugs on $\cdot\bar{O}_2$ and ·OH in vitro

Effect on $\cdot\bar{O}_2$ The ability of the drugs to react with $\cdot\bar{O}_2$ generated by the irradiated riboflavin system was used to evaluate the effectiveness of the drugs in scavenging $\cdot\bar{O}_2$. As illustrated in Figure 3a, NAC, Cys and cystamine all had no significant effect on scavenging $\cdot\bar{O}_2$.

Effect on ·OH The intensity of the ESR signal (which is proportional to the concentration of spin adducts in the sample) was determined by measuring the height of the first line of the second doublet of the spectrum and expressed in signal unit. As shown in Figure 3b, ESR signals characteristic of radical adducts of DMPO appeared in the control mixture (saline); however, DMPO adduct production in the mixture in which Cys, NAC, cystamine and mannitol were added was reduced to 2.18 ± 0.22 (Cys, $P < 0.05$ vs saline), 4.62 ± 0.85 (NAC), 4.01 ± 0.91 (cystamine) and 2.82 ± 0.47 (mannitol, $P < 0.05$ vs saline) respectively from 7.66 ± 1.32 (saline control). From this *in vitro* study, Cys scavenged ·OH significantly, however, both NAC and cystamine (at 3.6×10^{-4} M) did not have a significant scavenging effect on ·OH compared with the saline control ($P > 0.05$).

Discussion

The major finding in this study is that SH compounds, NAC and Cys which were administered before ischaemia and reperfusion, can improve the recovery of ventricular function in a rat model of stunned myocardium. This beneficial effect of NAC and Cys on stunned myocardium is probably through the following mechanisms.

(a) NAC and Cys do benefit the ischaemic and reperfused myocardium by significantly enhancing CFR during occlusion and reperfusion after a brief period of ischaemia. This may be the major mechanism by which NAC and Cys protect the myocardium against ischaemic and reperfusion injury. Winniford *et al.* (1986) have shown that NAC could potentiate the coronary vasodilator effect of nitroglycerin in man, as shown by an increase in coronary sinus blood flow. Blaustein *et al.* (1989) demonstrated that GSH administration could prevent an increase in coronary vascular resistance during 30 min of reperfusion after 20 min of ischaemia in isolated rat heart, suggesting that GSH may be particularly important in protecting the coronary vasculature. Other studies also show that some of the microvascular damage may be caused by free radicals because this damage is decreased by free radical scavengers (Przyklenk & Kloner, 1989; Zweier *et al.*, 1988). Conceivably, during a brief period of ischaemia and ensuing reperfusion, NAC and Cys may have a protective effect on both the myocardium and coronary vasculature through such mechanisms as: preserving or even potentiating coronary vasodilator reaction to some ischaemia-induced accumulated metabolites which can dilate coronary vessels; donating a SH group, which probably is involved in a common intermediate reaction through which all vasodilators act other than via 'specific receptors' (Needleman *et al.*, 1973), and replenishing endogenous GSH and/or scavenging ·OH.

The finding that NAC and Cys attenuated myocardial stunning through their actions in enhancing CFR was consistent with our previous reports in which a progressive decrease in CFR was found to be occurring in the early period of reperfusion following 20 min of global ischaemia and in which hyperosmotic mannitol, given on reperfusion, prevented this decrease and therefore reduced myocardial dysfunction (Sun & Lin, 1990; Sun *et al.*, 1991).

(b) The *in vitro* studies described in this paper demonstrate the ability of Cys to scavenge ·OH rather than $\cdot\bar{O}_2$. This suggests that the beneficial effects of Cys on stunned myocardium are due, at least in part, to prevention of ·OH production. This result is comparable with the reports by Bolli *et al.* (1987, 1988) and Faber *et al.* (1988), who have shown that the ·OH

radical or one of its reactive products is a mediator of post-ischaemic dysfunction.

NAC did not show a significant effect in scavenging ·OH ($P > 0.05$ vs saline) but it may exert its protective effects through mechanisms other than its beneficial effect on CFR, such as maintaining intracellular GSH concentration. NAC is a low molecular weight precursor of GSH and has been used successfully to replenish intracellular GSH stores in several diseased states (Olson *et al.*, 1980; Rumack *et al.*, 1981; Bernard *et al.*, 1984; Moldeus *et al.*, 1986). Furthermore, NAC may increase cytoplasmic superoxide dismutase activity and interfere with autocatalytic lipid peroxidation (Papaccio, 1986; Tribble *et al.*, 1987). Therefore, we suggest that NAC may work either through promoting the resynthesis of GSH or acting as an alternative substrate for glutathione peroxidases in this experimental model. These actions would limit the cytotoxic effects of free radicals and lipid peroxides.

(c) The chemical structure of three compounds, NAC, Cys and cystamine, were similar except that Cys and NAC have a free SH group but cystamine does not. However, the effects of NAC and Cys on myocardial stunning were significantly different from that of cystamine. Both NAC and Cys attenuated ventricular dysfunction in the postischaemic period whereas cystamine did not. This suggests that the free SH group is necessary in order to be effective in providing protection to the myocardium against ischaemia and reperfusion injury, particularly in improving coronary flow during ischaemia and reperfusion.

A number of studies in isolated perfused hearts (Woodward & Zakaria, 1985; Bernier *et al.*, 1986; Heuer *et al.*, 1988) and in rat models of transient regional ischaemia *in vivo* (Manning *et al.*, 1984; 1988) have supported the concept that oxygen free radicals play a role in the development of reperfusion arrhythmias. Superoxide dismutase, catalase, L-methionine, mannitol, GSH and desferrioxamine all reduced the incidence of reperfusion-induced VF (Bernier *et al.*, 1986). From our previous study (Tang & Tang, 1990), the antioxidant compounds, NAC and Cys, also showed beneficial effects in reducing the incidence of VF. However, in this experiment, although NAC and Cys showed a strong tendency to reduce reperfusion-induced VF, this effect did not reach statistical significance ($P > 0.05$ vs saline), which was probably due to the small size of the groups used in this study.

In summary, the data presented in this paper demonstrated that NAC and Cys improve the functional recovery of stunned myocardium. This ability was most likely due to their beneficial effect in preventing the fall in CFR on coronary occlusion and increase CFR on reperfusion, although they may also have a beneficial effect in scavenging ·OH and replenishing the intracellular GSH store. These protective effects appear to be correlated closely with their free SH group because when this group is replaced by a disulphide bond in the compound cystamine, the beneficial effect in the stunned myocardium was not observed. These results also suggest that the pathogenesis of myocardial stunning involves a multifactorial process; free radicals may play an important role in this process, however, other factors, such as coronary perfusion can greatly affect the recovery of postischaemic ventricular function as well.

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