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A direct protective effect of sulphinpyrazone on ischaemic and reperfused rat hearts

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1 Initiation of 60 min ischaemia to rat isolated hearts produced a depression in developed tension and heart rate. Subsequent reperfusion caused a greatly exacerbated creatine phosphokinase (CPK) efflux and limited functional recovery.

2 Sulphinpyrazone (100 ng ml^{-1} and 1 μg ml^{-1}) significantly reduced CPK release, particularly after reperfusion, the lower concentration being more effective.

3 A reduction in the mechanical depression during ischaemia and enhanced recovery after reperfusion were seen only with 100 ng ml^{-1} sulphinpyrazone. Heart rate and coronary perfusion pressure were unaffected by drug treatment.

4 The reduction in reperfusion-induced CPK efflux by 100 ng ml^{-1} sulphinpyrazone was maximal when the drug was present throughout the perfusion period although some protection was evident when sulphinpyrazone was present either during ischaemia or reperfusion only. An enhanced recovery in contractility was seen only when the drug was present throughout all phases of perfusion.

5 It is suggested that sulphinpyrazone exerts a direct protective effect on the heart particularly during reperfusion. The degree of protection is critically dependent on the concentration of sulphinpyrazone.

Introduction

Sulphinpyrazone (1,2-diphenyl-4-(2-phenyl-sulphinyl)ethyl)-3,5-pyrazolidiedione, Anturan) has been demonstrated in clinical trials to reduce sudden death after a second myocardial infarction (Anturan Reinfarction Trial Research Group, 1978; 1980). The mechanism of its beneficial effect is uncertain although it may be related to its ability to reduce platelet aggregation (Smythe *et al.*, 1965), a phenomenon seemingly dependent on platelet cyclooxygenase inhibition which would reduce thromboxane A_2 production (Ali & McDonald, 1977). Studies using experimental animal models have demonstrated that sulphinpyrazone protects the heart from various pathological insults including catecholamine administration (Karmazyn *et al.*, 1981a; Innes & Weisman, 1981) as well as coronary artery ligation (Brunner *et al.*, 1980; Innes & Weisman, 1981; Davenport *et al.*, 1981) *in vivo*, although the mechanistic basis for this protection still remains obscure. Few studies have been done on *in vitro* preparations in which blood-borne agents such as platelets would not be contributing factors. The present study was carried out in order to determine any possible direct effects of sulphinpyrazone on the rat isolated heart made ischaemic for 60 min followed by up to 60 min of reperfusion.

Methods

Animals

All experiments were carried out on male Sprague-Dawley rats (average weight 250 g) obtained from Canadian Breeding Laboratories, St. Constant, Quebec, Canada. The animals were housed 2 per cage on a 12 h light/12 h dark cycle until the day of the experiments and were given access to standard rat chow and water *ad libitum*.

Heart perfusion

The animals were killed by decapitation and their hearts were rapidly excised and placed in ice-cold Krebs-Henseleit solution. They were then picked up by the aorta with fine-tip forceps and mounted on a

steel perfusion cannula for perfusion via the coronary arteries according to the Langendorff method. The hearts were perfused by constant flow with a Watson-Marlow MHRE100 peristaltic pump. The perfusion fluid was a Krebs-Henseleit solution containing (mM) NaCl 120, $NaHCO_3$ 20, KCl 4.63, KH_2PO_4 1.17, $MgCl_2$ 1.20, $CaCl_2$ 1.25 and glucose 8; pH 7.4.

The hearts were initially perfused at 10 ml min^{-1} for 30 min. After this initial equilibrium period, global ischaemia was produced by reducing the flow rate to 1 ml min^{-1} for 60 min after which the initial flow rate was resumed for a further 15 min. Some experiments were also done in which the reperfusion period was extended to 60 min as described in the Results section. The entire perfusion system was thermoregulated at 37°C.

After commencing perfusion the apex of the heart was attached to a Grass FT.03 force-displacement transducer to record the myocardial contractile force. The transducer was adjusted to yield an initial resting tension of 2 g and was not changed thereafter. The transducer signal was connected to an analog differentiator to obtain $\delta F/\delta t$. A side arm off the perfusion cannula was connected to a Statham pressure transducer for obtaining the coronary perfusion pressure. Since the hearts were perfused at a constant flow, changes in coronary pressure were regarded as indicative of changes in coronary vascular resistance. All recordings were made on a Grass Model 7 polygraph.

Measurement of creatinine phosphokinase (CPK) leakage

CPK levels were measured in the cardiac effluent during various stages of the experiment with kits obtained from Sigma (St. Louis, MO.) on a Pye-Unicam Spectrophotometer.

Drugs

Sulphinpyrazone (Anturan) was a gift from Geigy Pharmaceuticals (Toronto, Ontario, Canada). It was

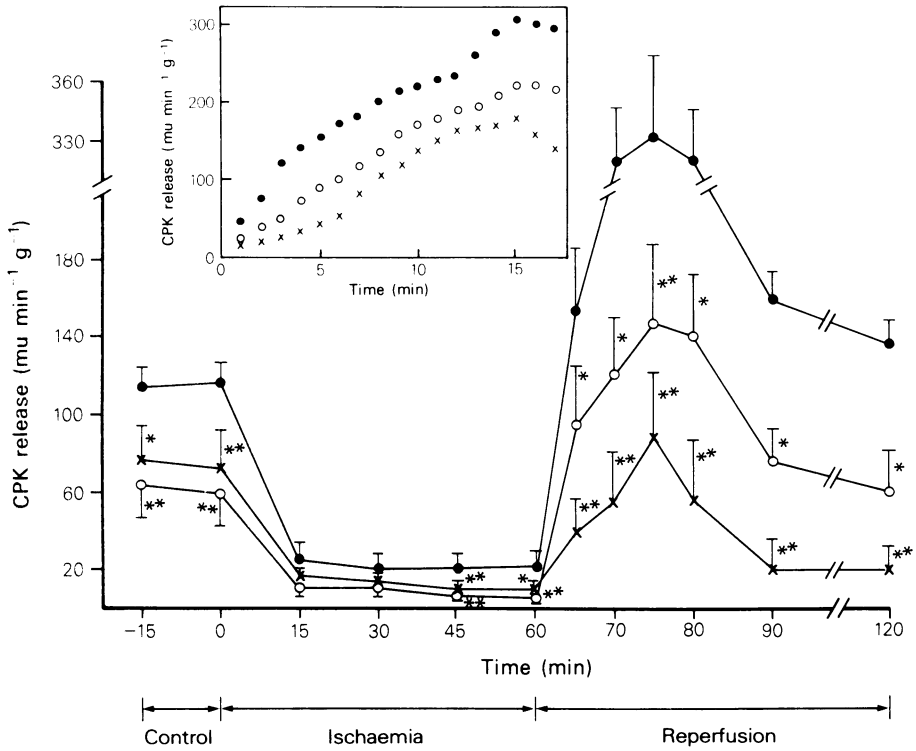

Figure 1 Creatine phosphokinase (CPK) release profile under control, ischaemic and reperfusion conditions. Hearts were treated either with no drug (untreated) (●, $n = 9$), 100 ng ml^{-1} sulphinpyrazone (X, $n = 12$) or 1 μg ml^{-1} sulphinpyrazone (○, $n = 8$). Note that the number of observations is reduced ($n = 3$) after 75 min perfusion (15 min reperfusion). Points represent means and vertical lines represent s.e.mean. * $P < 0.05$; ** $P < 0.01$, different from untreated values. Inset depicts CPK profile with values obtained every minute for 17 min of one heart each under different treatments. Symbols as described above.

neutralized with NaOH and dissolved in 0.9% w/v NaCl solution before addition to the perfusion medium. The drug was added during various stages of the experimental procedure (see Results). Appropriate control experiments were carried out with the sulphinpyrazone vehicle only. Vehicle concentrations in the buffer never exceeded 0.01%.

Statistical analysis

Comparisons between different treatment groups were made by analysis of variance with a Student-Newman-Keuls test to determine significant differences between groups.

Results

The initiation of myocardial ischaemia followed by reperfusion is manifested by several distinct changes. The initial reduction in coronary flow is characterized by loss of enzyme, and depression of contractility and heart rate. Reperfusion following 60 min of ischaemia results in incomplete recovery of function which is accompanied by an exacerbated enzyme efflux.

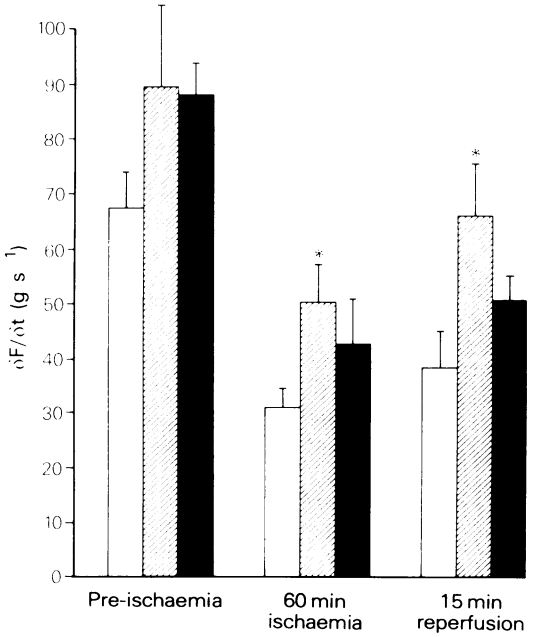

Figure 2 Mean values of $\delta F/\delta t$ before ischaemia, 60 min after initiating ischaemia and 15 min post-reperfusion in untreated hearts (controls: open columns, $n = 6$) and in hearts treated with sulphinpyrazone 100 ng ml^{-1} (hatched columns, $n = 9$) and 1 μg ml^{-1} (solid columns, $n = 5$). Vertical lines represent s.e.mean. * $P < 0.05$, different from control.

Figure 1 depicts the profile of CPK release during control perfusion, ischaemia and reperfusion. It should be noted that although the rate of CPK efflux actually falls during ischaemia this reflects a 90% reduction in coronary flow and actual CPK concentration in the effluent was substantially enhanced. Reperfusion produced an approximately three fold elevation in CPK as compared to pre-ischaemic values which peaked between 10 and 15 min. Each of three hearts was also studied under various treatments in which reperfusion was extended to 60 min. As shown in Figure 1, CPK release fell after 15 min of reperfusion. Although both sulphinpyrazone concentrations reduced CPK efflux throughout perfusion, maximum reduction was evident with 100 ng ml^{-1} and this effect was especially significant during reperfusion.

It is also noteworthy that in hearts perfused with 10 ng ml^{-1} sulphinpyrazone, CPK values tended to be lower than pre-ischaemic values after 60 min of reperfusion whereas in control hearts CPK release tended to be higher at this point. These differences were not statistically significant, probably owing to

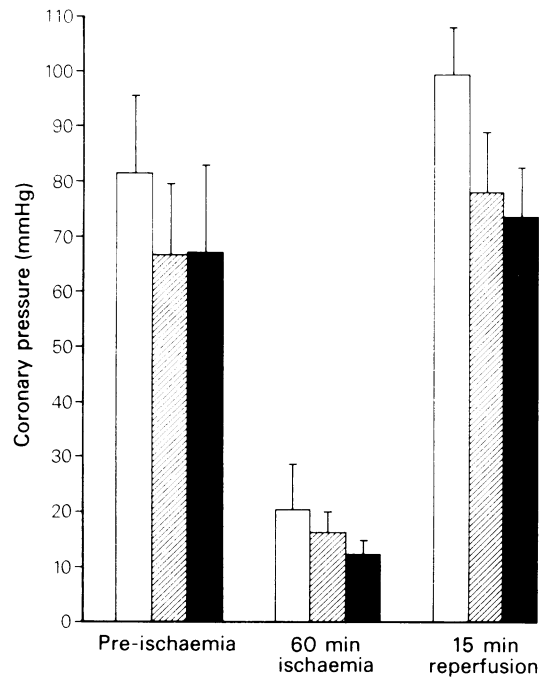

Figure 3 Mean coronary pressure values before ischaemia, 60 min after initiating ischaemia and 15 min post-reperfusion in untreated hearts (open columns, $n = 6$) and in hearts treated with sulphinpyrazone 100 ng ml^{-1} (hatched columns, $n = 9$) and 1 μg ml^{-1} (solid columns, $n = 5$). Vertical lines represent s.e.mean. There were no significant differences between any group.

the small number of samples used to obtain the values after 60 min of reperfusion. Figure 1 also shows one example of the CPK efflux determined at each minute following reperfusion (up to 17 min) from hearts under different treatments (inset). These values demonstrate that CPK release was reduced throughout the reperfusion period.

Figure 2 summarizes the contractility data. Sulphinpyrazone by itself increased $\delta F/\delta t$ when added before ischaemia although there was no significant overall differences between treatment groups. At the termination of the ischaemic period, $\delta F/\delta t$ of control hearts fell by more than 50% and was not substantially restored following reperfusion. In contrast, in the presence of 100 ng ml^{-1} sulphinpyrazone, the degree of myocardial depression was less and $\delta F/\delta t$ recovered to about 75% of pre-ischaemic values (as compared to 55% for control hearts). There were no significant differences in contractility between control hearts and hearts treated with 1 μg ml^{-1} sulphinpyrazone. It should be noted that all hearts demonstrated maximum recovery of mechanical function and rate of beating 10–15 min following reperfusion. Thus, in those hearts that were reperfused for extended periods no further recovery was observed. Average heart rates (± s.e.mean) before ischaemia were 324 ± 26, 291 ± 31 and 318 ± 38 beats min^{-1} for control hearts and hearts treated with 100 ng ml^{-1} and 1 μg ml^{-1} sulphinpyrazone, respectively. Ischaemia reduced heart rate by about 50% whereas all heart rates returned to about 80% of pre-ischaemic values following reperfusion. At no time were there significant differences between heart rates. Although with sulphinpyrazone coronary pressure tended to be lower reflecting a direct effect of the drug, these differences were not statistically significant (Figure 3).

A series of experiments was performed to determine during which phase of perfusion 100 ng ml^{-1} sulphinpyrazone acted to reduce CPK release and enhance mechanical recovery upon reperfusion (Figure 4). With respect to CPK inhibition the best

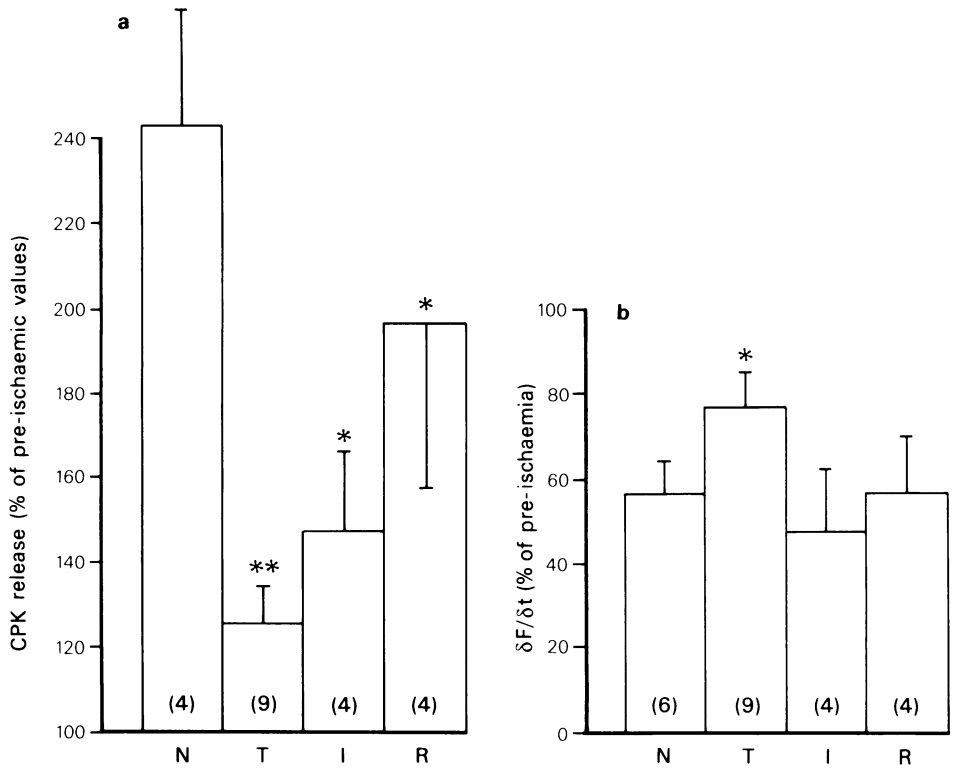

Figure 4 An analysis of the protection afforded by 100 ng ml^{-1} sulphinpyrazone against reperfusion injury when present during different stages of perfusion. (a) Depicts the % increase in creatine phosphokinase (CPK) release from pre-ischaemic values. (b) Demonstrates the % recovery in $\delta F/\delta t$ after reperfusion. Each column represents the mean values obtained 15 min after starting reperfusion and vertical lines show s.e.mean. N, no drug (control); T, 100 ng ml^{-1} sulphinpyrazone throughout perfusion; I, 100 ng ml^{-1} sulphinpyrazone during ischaemia only and R, 100 ng ml^{-1} sulphinopyrazone during reperfusion only. * $P<0.05$; ** $P<0.01$, different from control values.

protection was seen when the drug was present throughout the perfusion period. Substantial reduction in enzyme release was also evident when sulphinpyrazone was present during ischaemia. When the drug was added only during ischaemia there was still a significant decrease in CPK release, although this effect was much less than that seen in the other 2 treatment groups. In contrast to the CPK results, enhanced mechanical recovery on reperfusion occurred only when sulphinpyrazone was present during ischaemia as well as reperfusion.

Discussion

Clinical trials with sulphinpyrazone have demonstrated protection against sudden death in patients suffering a secondary myocardial infarction (Anturan Reinfarction Trial Research Group, 1978; 1980). The mechanism of this beneficial effect is uncertain although these trials were carried out because sulphinpyrazone is a potent inhibitor of platelet aggregation. In experimental animals sulphinpyrazone has been shown to protect against cardiac injury produced by coronary artery ligation by increasing collateral blood flow (Davenport *et al.*, 1981), reducing mortality (Brunner *et al.*, 1980) and preserving histological integrity (Innes & Weismann, 1981). Sulphinpyrazone also protects against catecholamine-induced cardiac injury as manifested by a lower mortality, reduced cardiac enzyme release, maintenance of histological integrity and prevention of coronary vasoconstriction (Karmazyn *et al.*, 1981a,b). Furthermore, arrhythmias produced by such methods as myocardial reperfusion (Povalski *et al.*, 1980) or adrenochrome administration (Beamish *et al.*, 1981) *in vivo* are reduced by sulphinpyrazone treatment. In spite of these findings the mechanism for the protective effects of sulphinpyrazone still remains obscure. Although inhibition of platelet aggregation may be a contributory factor, a recent study has demonstrated that sulphinpyrazone fails to protect the ischaemic myocardium of dog at concentrations that inhibit the aggregation of platelets (Bolli *et al.*, 1981) suggesting the involvement of other mechanisms.

The above studies demonstrate a protective effect of sulphinpyrazone *in situ* which may involve either direct actions on the heart or indirect mechanism such as inhibition of platelet aggregation or against drug-induced damage. The present study was carried out in order to determine whether sulphinpyrazone has any direct protective effect on the heart when blood-borne factors such as platelets are excluded.

Two types of heart damage were assessed; the initial insult as a consequence of flow reduction and the exacerbation of injury seen on reperfusion (Hearse, 1977). A number of overall conclusions can be reached. At $100\,ng\,ml^{-1}$, sulphinpyrazone 'protects' the ischaemic and reperfused myocardium. This property is manifested by a reduction of CPK release especially upon reperfusion, a reduction in the mechanical depression at the end of ischaemia and an enhancement of contractile recovery following reperfusion. The effect of suphinpyrazone was clearly concentration-dependent; with $1\,\mu g\,ml^{-1}$ there was less influence although beneficial effects on CPK release were still evident. Thus, these results also demonstrate dissociation between inhibition of CPK release and enhanced mechanical activity. Although enzyme release upon reperfusion was significantly reduced irrespective of which stage the drug was present, an enhanced recovery of contractility was evident only when sulphinpyrazone was present throughout the perfusion period. Such a discrepancy between CPK release and functional recovery using another model of heart damage has been found previously (Digerness *et al.*, 1980). An important point to emphasize from the present study is that sulphinpyrazone's salutary actions are not concentration-dependent, the lower amount being more effective, especially in decreasing reperfusion-induced dysfunction. Indeed, although not shown in this paper, all protective actions of sulphinpyrazone on CPK efflux are abolished at $10\,\mu g\,ml^{-1}$ and the drug causes a substantial depression in contractility at this very high concentration. This emphasizes the complexity of the properties of this drug as well as indicating that critical concentrations are required to achieve optimum cardio-protection.

An obvious question arises as to the possible mechanism of action of sulphinpyrazone. Clearly platelet factors cannot be involved since the perfusion medium was totally devoid of any blood components. Although coronary perfusion pressure was generally lower in the presence of sulphinpyrazone there were no significant differences between any treatment groups and therefore it is unlikely that any vasodilatation was contributing towards its protective effect. A likely possibility is that sulphinpyrazone exerted a direct cytoprotective effect. How this is accomplished is uncertain although it is quite possible that sulphinpyrazone has the ability to exert some degree of membrane stabilization thereby reducing CPK (a membrane bound enzyme) efflux during ischaemia and reperfusion. Such possible cellular and subcellular associated phenomena of cardio-protective agents including sulphinpyrazone are currently being studied in this laboratory.

Extrapolation of the present results to the clinical situation should be done cautiously. Nevertheless, it is noteworthy that the salutary effects observed in this study were at concentrations well within those seen in human plasma (Maguire *et al.*, 1981; Peder-

son *et al.*, 1982). It may be possible that *in vivo*, sulphinpyrazone directly protects and enhances the salvage of injured myocardium or at least that this property contributes to the overall protective action of this drug.

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