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Protective effects of ranolazine in guinea-pig hearts during low-flow ischaemia and their association with increases in active pyruvate dehydrogenase

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1 In isolated Langendorff-perfused, electrically-paced, hearts of guinea-pigs, global low-flow-ischaemia (LFI; at 0.7 ml min^{-1}) resulted in marked increases in the rates of release of lactate, lactate dehydrogenase (LDH) and creatine kinase (CK) over a 30 min period. At the end of the LFI period, tissue ATP content was significantly reduced from a control value of 11.8 ± 0.8 (5) to 5.6 ± 0.8 (5) $\mu mol\ g^{-1}$ dry weight.

2 The presence of ranolazine [(±)-N-(2,6-dimethyl-phenyl)-4[2-hydroxy-3-(2-methoxy-phenoxyl)-propyl]-1-piperazine acetamide dihydro-chloride; RS-43285-193] at 10 μM, from 20 min prior to and during LFI, resulted in significant reductions in the release of lactate, LDH and CK during the ischaemic period and a significant preservation of tissue ATP (9.0 ± 1.1 (6) $\mu mol\ g^{-1}$ dry wt.). Ranolazine did not prevent the reductions in creatine phosphate or glycogen observed in LFI, nor did it have any significant effects on any contractile parameters before or during the LFI period.

3 Neither ranolazine nor LFI affected the total amounts of tissue pyruvate dehydrogenase (PDH) activity; however, the significant reduction in the amount of active, non-phosphorylated PDH caused by LFI (from 88.2 ± 5.5 to 44.2 ± 3.2% of total activity) was partially but significantly prevented by ranolazine (67.2 ± 6.8%). This effect of ranolazine on PDH may be part of the mechanism whereby the compound reduces lactate release and preserves tissue ATP during ischaemia.

Keywords: Ranolazine; ischaemia; pyruvate dehydrogenase; guinea-pig heart

Introduction

The novel anti-anginal agent, ranolazine [(±)-N-(2,6-dimethyl-phenyl)-4[2-hydroxy-3(2-methoxyphenoxy)propyl]-1-piperazine acetamide di-hydrochloride; RS-43285-193] (Jain *et al.*, 1990; Cocco *et al.*, 1992) has been shown to be effective in inhibiting the biochemical and gross electrocardiographical consequences of transient myocardial ischaemia in the intact dog (Allely *et al.*, 1987; Allely & Alps, 1988) without causing direct haemodynamic effects. Ranolazine has also been shown to be remarkably effective in preventing myocardial enzyme release in an intact primate model of ischaemia with reperfusion (Allely & Alps, 1990). It has been suggested that ranolazine may alleviate the symptoms of transient myocardial ischaemia by altering substrate utilization (Allely *et al.*, 1987). The present study was undertaken to characterize the effects of ranolazine in an *in vitro* model of ischaemia, the guinea-pig isolated heart subjected to low-flow perfusion, in order to obtain direct biochemical evidence of cardioprotection, and also to gain some insights into the mechanism(s) underlying the anti-ischaemic properties of ranolazine. A small part of the present work has been previously reported briefly as a Meeting abstract (Clarke *et al.*, 1992).

Methods

Hearts from female Dunkin-Hartley guinea-pig (200–400 g) were retrogradely perfused (Langendorff method) at ~9 ml min^{-1} with a modified Krebs/bicarbonate solution containing 11.7 mM glucose and 1.9 mM $CaCl_2$ and gased with O_2/CO_2 (19/1) (normoxic control conditions); hearts were electrically-paced throughout at 250–300 beats min^{-1} (~25% above control intrinsic rate in each case). Each preparation was maintained at 37°C within a humidified chamber and allowed to equilibrate for 30 min prior to a pretreatment period of 20 min with or without 10 μM ranolazine. Low-flow-ischaemia (LFI) (± ranolazine) was induced by reducing the perfusion flow rate to 0.7 ml min^{-1} for 30 min; normoxic controls were perfused for 80 min as given above. Perfusate was collected at 2 min intervals for subsequent analysis of lactate, lactate dehydrogenase (LDH) and creatine kinase (CK) release, and then at the end of the perfusions the hearts were freeze-clamped using Wollenberg clamps pre-cooled in liquid N_2 before subsequent analysis of adenosine-5′-triphosphate (ATP), creatine phosphate and glycogen contents; all of these assays were performed with standard commercial kits, except for glycogen which was assayed by the method of Good *et al.* (1933). Samples were extracted and analysed for both active, non-phosphorylated, pyruvate dehydrogenase (PDH_a) activity and total PDH activity as described in McCormack & Denton (1989). A unit of enzyme activity is defined as that which converts 1 μmol of substrate min^{-1} at 30°C. Statistical analysis was by unpaired Student's *t* test and by analysis of variance and application of Dunnett's *t* test.

Results

Figure 1 (a–c) shows that LFI caused marked increases in the efflux of lactate, LDH and CK respectively from untreated hearts, and thus clearly shows that the present protocol is suitable for testing cardioprotection to ischaemic insult. We chose to study the effects of a concentration of ranolazine (10 μM) which had previously been shown to give maximal protection against several indices of ischaemic damage in *in vitro* rat heart preparations (Ferrandon *et al.*, 1988; 1990). Ranolazine reduced the magnitude of all of the presently used indices of damage during LFI (Figure 1), and

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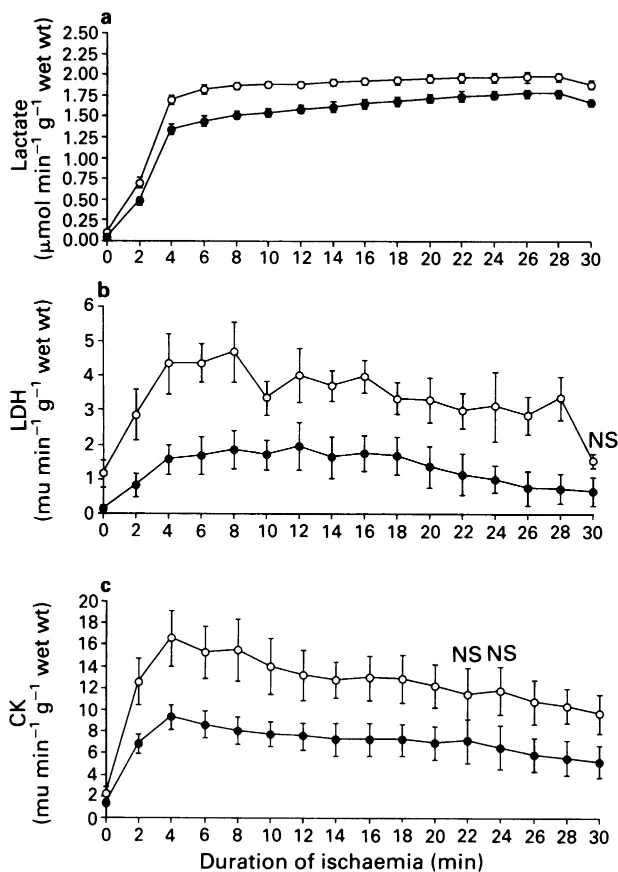

Figure 1 Effects of ranolazine on (a) lactate efflux and the release of (b) lactate dehydrogenase (LDH) and (c) creatine kinase (CK) from the perfused guinea-pig heart during a period of low-flow ischaemia. Values are means ± s.e.mean for 5 (untreated) or 6 (ranolazine-treated) hearts; g wet wt = g wet weight. The effects of 10 μM ranolazine (●) were significantly different ($P \leqslant$ at least 0.05) from the untreated controls (○) at all time points other than those (three) marked NS.

in all but 3 out of 45 instances (as indicated) significant differences from untreated control values (P at least $\leqslant 0.05$) were obtained. Table 1 shows that LFI resulted in a decrease in the tissue contents of ATP, creatine phosphate and glycogen at the end of the perfusions, and also a decrease in the amount of active PDH. Ranolazine also prevented the decrease in tissue ATP but did not affect the creatine phosphate or glycogen contents. Ranolazine largely prevented the LFI-induced decrease in active PDH; neither ranolazine nor LFI affected the total amount of PDH which ranged from 28 to 49 units g^{-1} dry weight. Ranolazine was without effect on contractile function either in normoxia or in LFI (results not shown).

Discussion

This study shows that ranolazine can prevent some of the deleterious consequences of cardiac ischaemia in an *in vitro* isolated heart model. This is supportive of the reports of its cardioprotective effects seen *in vivo* (see Introduction), and *in vitro* in rat heart preparations where several other different indices of cell damage were measured (Ferrandon *et al.*, 1988; 1990). The present demonstration of the preservation of tissue ATP content is particularly noteworthy, and may offer at least partial explanation for the improvement in functional parameters on reperfusion following ischaemia noted in some earlier studies.

PDH occupies a key site in respiratory fuel selection, effectively dictating the rate of carbohydrate utilization, and is subject to very complex regulation by many different factors (see Randle, 1986). There are not many reports of PDH measurement in guinea-pig heart (compared to rat heart for instance), however, the values obtained for total activity in the present study are similar to those found previously (e.g. Bunger *et al.*, 1983). The control values of PDH_a obtained in the present study appear to be fairly high as a % of total activity (see McCormack & Denton, 1989); however, they are again similar to those found in an earlier study on glucose-perfused guinea-pig hearts electrically paced at the relatively high frequency used (Hansford *et al.*, 1990).

In rat heart, ischaemia or reperfusion have previously been shown to lead to an inactivation of PDH (Kobayashi & Neely, 1983; Patel & Olson, 1984) under conditions similar to those used in the present study, which indicates that a similar situation may occur in the guinea-pig. Ischaemia is likely to give rise to increased intramitochondrial $NADH/NAD^+$ and acetyl CoA/CoA ratios which would lead to complex inactivation; however, the reduced ATP/ADP would balance this to some extent by favouring activation (see Kobayashi & Neely, 1983). In this context it is worth noting that ranolazine preserves tissue ATP in ischaemia yet is associated with PDH activation.

The anti-ischaemic mechanism of ranolazine appears to differ from many commonly prescribed anti-anginal agents (e.g. β-blockers, calcium channel blockers) in that haemodynamics appear to be unaffected (Allely & Alps, 1988; 1989). Although it is not clear whether the activation (or prevention of ischaemic inhibition) of PDH is a part of the cause, or a consequence of the cardioprotective or anti-anginal mechanism, an attractive hypothesis can be built around the former possibility. An activation of PDH could result in a decrease in lactate release by allowing more lactate to be used as a substrate, and could also account at least to some degree for enhanced ATP production per unit of O_2 by favouring carbohydrate oxidation compared to fat (see e.g. Hutter *et al.*, 1985). This of course would be most beneficial under times of O_2 limitation such as reduced coronary flow and/or increased work demand, and may lower the delivered oxygen content at which biochemical and mechanical evidence of ischaemia develop (Burkhoff *et al.*, 1991). Tissue ATP preservation and perhaps reduced lactic acidosis may

Table 1 Effects of low-flow ischaemia (LFI) and ranolazine on the ATP, creatine phosphate (CrP), glycogen and active pyruvate dehydrogenase (PDH_a) contents of guinea-pig heart

Parameter	*Normoxia* (5)	*LFI* (5)	*LFI + ranolazine* (6)
ATP	11.8 ± 0.8	5.6 ± 0.8**	9.0 ± 1.1†
CrP	10.4 ± 0.1	1.9 ± 0.3**	2.3 ± 0.3**
Glycogen	92.9 ± 10.9	38.9 ± 5.0**	43.0 ± 3.0**
PDH_a	88.2 ± 5.5	44.2 ± 3.2*	67.2 ± 6.8†

Ranolazine was present at 10 μM; see methods for experimental conditions. The number of hearts for each condition is given in parentheses. Metabolite values are expressed in μmol g^{-1} dry weight; PDH_a values are expressed as % of total PDH.
Significant effects of LFI compared to the appropriate normoxic controls are indicated by $^*P \leqslant 0.01$, and $^{**}P \leqslant 0.001$, and significant effects of ranolazine versus the LFI untreated group are indicated by $^\dagger P \leqslant 0.05$.

thus allow more preservation of cellular viability and hence account for the observed effects on LDH and CK release as indicators of ischaemic cell damage.

The present observations offer some insights into potential mechanisms for the cardioprotective and anti-anginal effects of ranolazine. It will be important to see whether the drug has direct effects on PDH or whether the described effects are an indirect consequence of its affecting metabolism elsewhere; e.g. inhibition of fat oxidation would lead to activation of PDH (Randle, 1986).

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