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Prenylamine and the myocardial response to ischaemia and reperfusion: effects of acute and chronic treatment

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The ability of prenylamine gluconate (Segontin) to influence the extent of myocardial ischaemic injury was investigated in the isolated 'working' rat heart preparation. The drug was administered either acutely alone (4 μM litre^{-1} in the perfusion medium) or chronically plus acutely in which case animals were pre-treated (10 mg kg^{-1} day^{-1} orally) for 10 days and the drug was then also added (4 μM litre^{-1}) to the perfusate. Acute administration alone resulted in a small reduction in spontaneous functional performance of the aerobic isolated heart in comparison with gluconate treated controls. It also increased the percentage of hearts able to recover functional activity after a period of severe ischaemia and decreased ischaemia induced injury as assessed by enzyme leakage. In contrast to the acute results the combination of chronic and acute administration of prenylamine did not significantly alter spontaneous cardiac function. Although a small increase in the number of hearts that recovered function was apparent, there was a concomitant decrease in post-ischaemic functional performance with no reduction of ischaemia induced enzyme leakage.

Prenylamine (3'3' di-phenylpropyl-1-methyl-phenethylamine) has been available since 1960 for the treatment of coronary heart disease. It has been suggested that this agent may act at the level of the central nervous system, at the cardiac level and also on the peripheral vascular system. Pharmacological studies of the compound have suggested that it may inhibit sympathetic over-activity, that it may inhibit transmembrane calcium fluxes and that it may possess antiarrhythmic and negative chronotropic properties (Lindner 1973; Marshall & Parratt 1977).

We have used an isolated 'working' rat heart preparation in an attempt to distinguish some of the direct cardiac effects of prenylamine from the peripheral effects and also to investigate the extent to which both chronic and acute treatment with this compound might reduce ischaemic injury. A preliminary report of some of these results has been published (Manning et al 1981a).

METHODS

Animals and chronic drug administration

Male rats (280–320 g) of the Wistar strain were used for all studies. In the chronically treated series prenylamine gluconate was administered orally for 10 days by spraying food with an aqueous solution of the drug. In the control series, animals were given food which had been sprayed with an aqueous solution containing the appropriate amount of gluconate. To ensure that all the drug was consumed by the animals the normal daily food allowance was slightly reduced during the period of treatment.

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Perfusion techniques

Rats were lightly anaesthetized with diethyl ether, the right femoral vein was exposed and heparin (200 U) was administered intravenously. One minute later the heart was excised and was placed in ice cold perfusion medium until contraction had ceased (approximately 30 s). The aorta and left atrium were cannulated and the hearts perfused in the working mode as described by Neely et al (1967).

In this preparation the atrial filling pressure was equivalent to 20 cm H_2O and the hydrostatic afterload was 100 cm H_2O. Under these conditions the control hearts spontaneously ejected approximately 70 ml min^{-1}g^{-1} wet wt via the aorta, with a coronary flow of approximately 25 ml min^{-1}g^{-1} wet wt. During aerobic perfusion the following indices of cardiac function were recorded; heart rate, aortic pressure, aortic flow, coronary flow and cardiac output. After a 20 min aerobic, working, control period the hearts were made ischaemic by clamping the atrial inflow line and the aortic outflow line and then initiating (via a sidearm of the aortic cannula) a controlled retrograde flow equivalent to 0·14 ml min^{-1}g^{-1} wet wt. This was achieved using a constant flow infusion

pump. To ensure that this low flow infusion was delivered to the heart at 37 °C a heated cannula (Manning et al 1980a) was utilized. This prevented any cooling of the perfusate during its delivery to the heart.

Perfusion medium

Bicarbonate buffer, pH 7·4 (Krebs & Henseleit 1932; Hearse et al 1978) was the standard perfusion fluid. Glucose (11·1 mmol litre^{-1}) was included as a substrate and the perfusion fluid was gassed with 95% O_2 and 5% CO_2 ($\dot{P}o_2$ > 600 mm Hg). In both chronic and acute studies 4 μmol litre^{-1} prenylamine gluconate was added to the perfusion medium and in the control series gluconate was added at the appropriate concentration.

Perfusion time sequence

Immediately after mounting, the hearts were subjected to a 5 min washout in the Langendorff (1895) mode. The preparation was then converted to a working system for 20 min during which the various indices of cardiac function were recorded at 4 min intervals and a mean value for control function calculated. The aortic and left atrial cannulae were then clamped and low flow coronary infusion initiated. After 35 min of ischaemia, aerobic Langendorff perfusion was reinstated for 15 min. During both the ischaemic and post-ischaemic Langendorff perfusion period the coronary effluent was separately collected at 4 °C and was then taken for the analysis of creatine kinase activity. Following the post-ischaemic Langendorff perfusion period the preparation was re-converted to the working mode and the recovery of cardiac function monitored for 20 min.

In certain series, at the end of the post-ischaemic recovery period, hearts were freeze-clamped using stainless steel tongs cooled to the temperature of liquid-nitrogen. The hearts were then taken for the analysis of adenosine triphosphate (ATP) and creatine phosphate (CP).

Metabolite and enzyme analysis and expression of results

The measurement of ATP, CP and creatine kinase was as described previously (Oliver 1965; Hearse et al 1977). The ATP and CP contents were expressed as μmol g^{-1} dry wt, and the total enzyme leakage as IU per perfusion period.

At least 6 hearts were used for each study and the results expressed as the mean ± the standard error of the mean.

RESULTS

Acute treatment series

(a) *Basal function.* In this series hearts were perfused aerobically with buffer containing either 4 μmol litre^{-1} prenylamine gluconate or 4 μmol litre^{-1} gluconate. The effect upon cardiac function is shown in Table 1. In general, the drug exerted a small depressant effect, such that aortic flow was reduced by 12 ml min^{-1} (approximately 20%, $P < 0.01$) and aortic pressure by approximately 11 cm H_2O ($P < 0.05$). In an additional series of studies a higher concentration of drug (20 μmol litre^{-1}) was added to the perfusion medium, but this was found to depress cardiac function completely such that the hearts were unable to pump against the 100 cm H_2O pressure head.

Table 1. The effect of acute prenylamine treatment (4·0 μmol litre^{-1}) upon basal cardiac function.

	Gluconate ± s.e.m. (n)	Prenylamine ± s.e.m. (n)
Heart rate (beats min^{-1})	281 ± 10 (7)	277 ± 11 (7)
Aortic pressure (cm H_2O)	180 ± 4 (7)	169 ± 2* (7)
$\partial P/\partial t$ max (cm H_2O s^{-1})	4762 ± 45 (7)	4166 ± 337 (7)
Aortic flow (ml min^{-1})	70 ± 2 (7)	58 ± 2** (7)
Coronary flow (ml min^{-1})	25 ± 1 (7)	28 ± 1 (7)
Total cardiac output (ml min^{-1})	95 ± 3 (7)	86 ± 3 (7)

* Indicates a significant difference (using the paired Student's *t*-test) from control values at the level of $P < 0.05$ and ** at the level of $P < 0.01$. (n) denotes the number of experiments.

(b) *Resistance to ischaemia.* Prenylamine gluconate was present in the treated group in both the aerobic and the ischaemic periods. In the control series, gluconate carrier (4 μmol litre^{-1}) was included during both periods. The effect upon enzyme leakage and post-ischaemic function and metabolism is detailed in Table 2. A protective effect of prenylamine was observed, with a significant improvement in aortic flow, coronary flow, cardiac output and enzyme leakage. This improvement however could be largely (but not entirely) attributed to a striking improvement in the number of hearts which successfully recovered from the ischaemic period. In the control group only two out of seven hearts recovered whereas in the drug treated group six of the seven hearts recovered. Again, no significant differences in tissue metabolite levels were detected.

Combined chronic and acute treatment series

Basal function. In this series of studies rats were treated with prenylamine gluconate (10 mg kg^{-1} day^{-1}) for 10 days and the drug was also included in the perfusion fluid (4 μmol litre^{-1}). In the control series gluconate was substituted during chronic and acute

Table 2. The effect of acute prenylamine treatment (4·0 μmol litre^{-1}) upon resistance to ischaemia as assessed by post ischaemic functional recovery, enzyme leakage and tissue metabolite levels.

	Gluconate ± s.e.m. (n)	Prenylamine ± s.e.m. (n)
No. hearts that recovered function	2/7	6/7
Creatine kinase leakage (IU) during ischaemia	0·16 ± 0·02 (7)	0·17 ± 0·02 (7)
Creatine kinase leakage (IU) during Langendorff reperfusion	10·9 ± 1·0 (7)	8·2 ± 0·5* (7)
ATP (μmol g^{-1} dry wt)	13·1 ± 0·5 (7)	14·5 ± 0·5 (7)
CP (μmol g^{-1} dry wt)	26·1 ± 4·0 (7)	27·9 ± 1·0 (7)
Observations in hearts that recovered		
Heart rate (beats min^{-1})	270 ± 0 (2)	250 ± 9 (6)
Aortic pressure (cm H_2)	138 ± 3 (2)	149 ± 3 (6)
$\partial P/\partial t$ max (cm H_2O s^{-1})	2381 ± 147 (2)	2827 ± 210 (6)
Aortic flow (ml min^{-1})	15 ± 1 (2)	22 ± 4 (6)
Coronary flow (ml min^{-1})	14 ± 1 (2)	18 ± 1* (6)
Total cardiac output (ml min^{-1})	28 ± 1 (2)	40 ± 2** (6)
Creatine kinase leakage (IU) during ischaemia	0·10 ± 0·01 (2)	0·18 ± 0·02 (6)
Creatine kinase leakage (IU) during Langendorff reperfusion	9·80 ± 1·31 (2)	8·12 ± 0·58 (6)
ATP (μmol g^{-1} dry wt)	16·1 ± 2·8 (2)	14·7 ± 0·6 (6)
CP (μmol g^{-1} dry wt)	35·6 ± 12·0 (2)	27·9 ± 1·2 (6)

* Indicates a statistical difference (Paired Student's *t*-test) from control values at the level of $P < 0.05$ and ** $P < 0.02$ (n) denotes the number of experiments.

treatment. No significant effects upon basal function were observed although in close agreement with the results in Table 1 there was a tendency to a slight depression of cardiac function.

Resistance to ischaemia. Table 3 reveals that the combination of chronic and acute treatment had a marked effect upon the number of hearts which recovered, thus while only 50% of the control hearts recovered, there was a 100% recovery in the drug treated group. Surprisingly, however, the absolute recoveries in the drug treated group were inferior to those of the control group with significant reductions in heart rate, aortic flow and coronary flow.

DISCUSSION

These results show that prenylamine, given acutely in doses which produce only a small reduction in basal cardiac function, can exert a number of protective effects in the isolated rat heart. These effects include a reduction in tisue damage (as measured by enzyme leakage) and an enhancement of post-ischaemic coronary flow and total cardiac output during post-ischaemic reperfusion. More strikingly, this compound increased significantly the number of hearts which recovered function following a period of severe ischaemia. When acute therapy was combined with long-term treatment the reduction in basal cardiac function was no longer observed and the reduction in enzyme leakage during reperfusion with acute treatment alone did not occur. The combination of acute and chronic therapy, however, still tended to increase the number of hearts able to resume pump function during the post-ischaemic phase (100% compared with 50% of the untreated group) but it was noted that hearts in the drug treated group exhibited a significantly lower level of functional activity than those hearts which recovered in the control group. This is in contrast with the effects observed during acute therapy alone. This chronic treatment regime has been shown previously to be beneficial to the ischaemic myocardium (Manning et al 1982) in producing an increase in post-ischaemic functional recovery and reducing enzyme leakage. Therefore, any affects of chronic therapy *alone* cannot be responsible for the reduction in post-ischaemic functional recovery. It is most likely that the reduced beneficial effects of combined acute and chronic therapy are due to the increased concentration of prenylamine available to the heart. This might indicate that the amount of protection afforded by prenylamine may not be directly proportional to the dosage used.

The mechanism underlying the protective effects is open to speculation but several distinct possibilities exist. Prenylamine is known to reduce endogenous catecholamine stores (Lindner 1973) and has been suggested (Hasselbach et al 1968) to reduce sudden transmembrane fluxes of calcium. These two actions would be expected to exert a protective effect against ischaemia and reperfusion induced tissue injury. An action upon transmembrane calcium fluxes or catecholamines would also be consistent with the observed slight cardio-depressant effects of the drug during aerobic perfusion.

Table 3. The effect of combined chronic (10 mg kg^{-1} day^{-1}) and acute (4·0 μmol litre^{-1}) prenylamine treatment upon resistance to ischaemia as assessed by post-ischaemic functional recovery, enzyme leakage and tissue metabolite levels.

	Gluconate s.e.m. (n)	Prenylamine s.e.m. (n)
No. hearts that recovered function	4/8	7/7
Creatine kinase leakage (IU) during ischaemia	0·25 ± 0·03 (8)	0·23 ± 0·02 (7)
Creatine kinase leakage (IU) during Langendorff reperfusion	8·5 ± 1·4 (8)	8·3 ± 0·9 (7)
Observations in hearts that recovered		
Heart rate (beats min^{-1})	277 ± 17 (4)	226 ± 11 (7)*
Aortic pressure (cm H_2O)	157 ± 8 (4)	151 ± 5 (7)
$\partial P/\partial t$ max (cm H_2O s^{-1})	4762 ± 595 (4)	4166 ± 338 (7)
Aortic flow (ml min^{-1})	38 ± 3 (4)	16 ± 5 (7)**
Coronary flow (ml min^{-1})	21 ± 1 (4)	16·6 ± 1·2 (7)*
Total cardiac output (ml min^{-1})	59 ± 3 (4)	32 ± 6 (7)**
Creatine kinase leakage (IU) during ischaemia	0·19 ± 0·03 (4)	0·23 ± 0·02 (7)
Creatine kinase leakage (IU) during Langendorff reperfusion	5·08 ± 1·16 (4)	8·33 ± 0·90 (7)

* $P < 0.05$, ** $P < 0.02$) (Paired Student's *t*-test) (n) denotes the number of experiments.

The protective effects of this drug are similar to those observed with β-blocking agents (Manning et al 1980b; Reimer et al 1973) and slow-channel calcium antagonists (Smith et al 1975; Henry et al 1977). In this connection calcium-antagonizing properties have been attributed to prenylamine (Fleckenstein et al 1977), however, it would seem most likely that the mode of action of these drugs is entirely different and as such they may all fulfil particular roles in the treatment of ischaemic heart disease.

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