Br. J. Pharmac. (1979), **65**, 479–482

STABILIZATION OF LYSOSOMES IN ANOXIC MYOCARDIUM BY PROPRANOLOL

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1 Isolated hearts of guinea-pigs were perfused aerobically and anoxically for 60 min. (±)-Propranolol was added to the perfusion medium in concentrations ranging from 10 ng to 10 μg/ml.

2 Lysosome stability was assessed by measurements of latent acid hydrolase activity in homogenates of left ventricular tissue.

3 In the absence of propranolol, the integrity of the myocardial lysosomes was considerably reduced after 60 min anoxia. Lysosome stability was enhanced by the presence of (±)-propranolol. The optimal concentration was found to be 0.1 μg/ml. This concentration of the (+)-isomer alone was less effective.

4 It was concluded that β-adrenoceptor blockade was the major mechanism by which lysosome disruption was prevented but that some direct membrane effect of propranolol may also be involved.

Introduction

The reduction of heart rate and contractility, with the consequent decrease in oxygen demand, produced by β-adrenoceptor blocking agents was considered to be the mechanism by which drugs such as propranolol reduced infarct size in experimental coronary artery occlusion (Moroko, Kjekshus, Sobel, Watanabe, Covell, Ross & Braunwald, 1971; Watanabe, Shintani, Fu, Fujii, Watanabe & Kato, 1972). However, Jennings has contested this assumption since a decrease in contractility could not be expected to protect cells in the central zone of ischaemia where contractile function has ceased (Jennings & Reimer, 1974). In studies with anoxically perfused non-contracting guinea-pig hearts Sakai & Spieckermann (1975) found that enzyme release could be reduced by treatment with reserpine or propranolol but their data show a greater degree of protection with propranolol. It seems likely, therefore, that propranolol may have a direct effect on myocardial cells which acts in addition to β-blockade in delaying the onset of necrosis in anoxia and ischaemia.

Propranolol is known to have a membrane stabilizing action (Barrett & Cullum, 1968; Fitzgerald, 1969) and it is possible that stabilization of myocardial cell membranes, particularly the lysosomal membranes, may prolong the viability of ischaemic cardiac muscle. This study was carried out to determine the effects of propranolol on lysosome stability in anoxically perfused guinea-pig heart. The roles of β-blockade and direct membrane stabilization were assessed by comparison of the effects of (±)-propranolol with those of the dextro-isomer. Only the laevo-isomer has significant β-blocking activity, while both isomers have the same degree of membrane activity (Barrett & Cullum, 1968).

Methods

Heart perfusion

Male Dunken-Hartley guinea-pigs (600 ± 50 g body wt.) were used for heart perfusion. The hearts were excised as described previously (Welman, 1974) and perfused at 37°C on a non-working system (Langendorff, 1895) incorporating the modifications of Hearse & Chain (1972). The perfusion fluid was a glucose-free Krebs bicarbonate buffer (Krebs & Henseleit, 1932) which was equilibrated with 95% O_2 and 5% CO_2 for aerobic perfusions and with 95% N_2 and 5% CO_2 in anoxic studies.

Propranolol was added to the perfusion fluid in concentrations ranging from 10 ng/ml to 10 μg/ml of either (±)-propranolol hydrochloride or (+)-propranolol hydrochloride (ICI).

Enzyme assays

The left ventricle tissue was removed after 60 min heart perfusion and a weighed sample was homogen-

0007-1188/79/030479-04 $01.00

ized with 10 vol sucrose 0.25 mol/l at 4°C in a Douncer homogenizer as described previously (Welman & Peters, 1977a). The homogenate was centrifuged at 600 *g* for 10 min to sediment the nuclei, myofibrils and unbroken cells. The post-nuclear supernatant (PNS-fraction) was assayed for protein (Lowry, Rosebrough, Farr & Randall, 1951) and for free and total acid hydrolase activity. Four lysosomal marker enzymes were measured, N-acetyl-β-glucosaminidase, N-acetyl-β-galactosaminidase, β-glucuronidase and cathepsin C. These enzymes are localized to different populations of lysosomes in myocardial tissue, which have been resolved by sucrose density gradient centrifugation (Welman & Peters, 1976). The enzyme assays were carried out with fluorogenic substrates, conjugates of 4-methylumbelliferone (Koch-Light Laboratories Limited) by the methods of Peters, Müller & de Duve (1972).

Latency

Estimation of lysosome integrity was made by calculation of the latency of the four marker enzymes. Latency was calculated from the free and total enzyme activities in the PNS-fraction thus:

$$\text{Latency} = \frac{\text{Total} - \text{Free}}{\text{Total}} \times 100\%.$$

Free enzyme activity was measured in the absence of a membrane disruptive agent, while total activity was assayed in the presence of digitonin (0.05 mg/ml) to release the bound enzymes.

Results

Aerobic perfusions

Control hearts perfused aerobically without drugs for 60 min showed no change in latency or in total acid hydrolase activity (mu/mg protein) when compared with non-perfused tissue (Welman, 1974). Inclusion of (±)-propranolol hydrochloride in the aerobic perfusion medium in concentrations of 0.1 μg/ml or 10 μg/ml did not alter these values. The mean latencies of the four lysosomal marker enzymes studied are shown in Table 1.

Anoxic perfusion

Hearts perfused anoxically for 60 min showed a marked decrease in the latency of each of the lysosomal enzymes without significant changes in total enzyme activity. These results are shown in Table 1. All the concentrations of (±)-propranolol tested (10 ng to 10 μg/ml) significantly increased the latency of N-acetyl-β-glucosaminidase and N-acetyl-β-galactos-

Table 1 Latencies of lysosomal enzymes in guinea-pig heart after 60 min perfusion with and without propranolol

	Latency (% total − free/total activity)			
Perfusion conditions	*N-acetyl-β-glucosaminidase*	*N-acetyl-β-galactosaminidase*	*β-Glucuronidase*	*Cathepsin C*
Aerobic: drug-free	66 (±4)	67 (±5)	29 (±5)	53 (±4)
Anoxic: drug-free (control)	36 (±5)	34 (±5)	14 (±4)	26 (±4)
(±)-Propranolol 10 ng/ml	*45 (±3)	*46 (±2)	**18 (±3)	**26 (±2)
(±)-Propranolol 100 ng/ml	*60 (±4)	*58 (±3)	*29 (±3)	*31 (±2)
(±)-Propranolol 1 μg/ml	*54 (±3)	*54 (±3)	*26 (±3)	*34 (±3)
(±)-Propranolol 2 μg/ml	*50 (±3)	*50 (±3)	*25 (±2)	**25 (±2)
(±)-Propranolol 10 μg/ml	*45 (±3)	*43 (±2)	**15 (±2)	**22 (±3)
(+)-Propranolol 10 ng/ml	**39 (±4)	**36 (±3)	**16 (±2)	**26 (±3)
(+)-Propranolol 100 ng/ml	*51 (±3)	*48 (±4)	*21 (±3)	**27 (±3)

Each value in the mean (± s.d.) of duplicate determinations on 6 hearts.
* $P < 0.001$; ** $P > 0.01$.

aminidase, markers for the major lysosomal population in myocardial cells (Welman & Peters, 1976). β-Glucuronidase, which is present in high density lysosomes as well as a lower density population had significantly more latent activity in the presence of 0.1 μg/ml to 2 μg/ml (±)-propranolol. Cathepsin C, which is present mainly in low density lysosomes, showed increased latency with 0.1 μg/ml and 1 μg/ml (±)-propranolol.

In contrast to the racemic drug, 10 ng/ml (+)-propranolol hydrochloride did not alter the latencies of the lysosomal enzymes in anoxic tissue. However, a significant stabilizing effect was observed with 0.1 μg/ml of the (+)-isomer for all the marker enzymes, except cathepsin C. The degree of effect of this concentration of (+)- propranolol was not as great as that found with the same concentration of the racemate.

Discussion

The results of this study show that (±)-propranolol can stabilize myocardial lysosomal membranes against the damaging effects of anoxia. The optimal concentration for this effect was found to be 0.1 μg/ml (±)-propranolol hydrochloride. This concentration is within the range used therapeutically for β-blockade (Shand, 1974). Higher concentrations of the drug reduced the latencies of the lysosomal marker enzymes and a similar effect has been observed with other lysosomal stabilizing agents (Welman & Peters, 1977b). It is possible that in some cases higher concentrations of these drugs may cause an increase in lysosomal size which could render the organelles more susceptible to disruption during homogenization of the tissue.

On the other hand, there is some evidence that high concentrations of propranolol may be less effective in protecting myocardial cells during anoxia. Hearse, Garlick, Humphrey & Shillingford (1978) using 2 μg/ml propranolol found only slight reduction in enzyme release from the perfused rat heart, while the low concentration used by Sakai & Speickerman (1975) considerably reduced enzyme release from perfused guinea-pig hearts.

The mechanism of the lysosome protection observed in this study appears to involve mainly β-blockade but also some direct effect of the drug since (+)-propranolol was found to stabilize partially the lysosomal membrane.

(+)-Propranolol has less than 1/50th of the β-blocking activity of the (−)-isomer (Barrett & Cullum, 1968) and it is therefore unlikely that (+)-propranolol could have any β-blocking activity at the concentrations tested. Both isomers of propranolol are known to have direct membrane effects which have been demonstrated by electrophysiological techniques. In concentrations of 10 μg/ml or above, both (+) and (−)-propranolol can reduce the rate of rise of the intracardiac action potential (Coltart, Meldrum & Hamer, 1970). Similar concentrations are associated with suppression of arrhythmias and local anaesthetic activity (Vaughan Williams, 1966). However, these quinidine-like effects are unlikely to be involved in the stabilization of lysosomal membranes unless the lysosomes can concentrate propranolol by at least 100 fold.

The role of lysosomal enzymes in myocardial cell death has yet to be established but disruption of the lysosomal membranes is one of the earliest irreversible changes that can be demonstrated in dying myocardial cells (Welman & Peters, 1977a; Decker, Poole, Griffin, Dingle & Wildenthal, 1977). Thus, the finding that propranolol enhances lysosome stability supports the conclusions of other investigators that this drug may be able to salvage ischaemic myocardium (Moroko *et al.*, 1971; Reimer, Rasmussen & Jennings, 1973). The exact mechanism by which this could be achieved remains uncertain but is likely to involve both β-blockade and a direct effect of the drug.

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(Received July 20, 1978.
Revised September 27, 1978.)