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THE EFFECT OF CHEMICAL SYMPATHECTOMY ON MITOCHONDRIAL FUNCTION IN THE ISCHAEMIC AND REPERFUSED MYOCARDIUM

WINIFRED G. NAYLER & ELIZABETH M. SCOTT[1]*

Department of Medicine, University of Melbourne, Austin Hospital, Heidelberg, 3084, Victoria, Australia and The Cardiothoracic Institute*, 2 Beaumont Street, London, W1N 2DX

1 Isolated rabbit hearts were perfused aerobically for 120 min, made ischaemic for 90 min, or made ischaemic for 90 min and then reperfused for 30 min.

2 Some rabbits were pretreated with 6-hydroxydopamine (6-OHDA), given as three separate intravenous doses of 30, 20 and 20 mg/kg, 20 to 48 h before they were killed; others (controls) received saline according to the same regime.

3 Mitochondria were harvested from left ventricular homogenates and their function assessed by measuring state 3 O_2 consumption (state 3 QO_2), respiratory control index (RCI), phosphate: oxygen ratio (ADP:O), Ca^{2+} content, and ATP-producing activity. In other experiments peak left ventricular developed tension was recorded.

4 In hearts from saline-treated animals, mitochondrial state 3 QO_2, RCI and ATP producing activities were reduced after global ischaemia, with or without reperfusion. There was a small gain in mitochondrial Ca^{2+} after ischaemia, and a large gain upon reperfusion.

5 6-OHDA pretreatment provided some protection against the effects of ischaemia and reperfusion on mitochondrial function and on peak developed tension.

6 It was concluded that chemical sympathectomy with 6-OHDA does not duplicate the effect of prolonged β-adrenoceptor blockade in protecting mitochondrial function against the deleterious effects of ischaemia and reperfusion.

Introduction

β-Adrenoceptor blockade has been shown to protect the myocardium against the damage caused by prolonged episodes of ischaemia and hypoxia, irrespective of whether these episodes occur naturally (Wilhelmsson, Vedin, Wilhelmsen, Tibblin & Werko, 1974; Norris, Clarke, Sammel, Smith & Williams, 1976; Yusef, Ramsdale, Peto, Furse, Bennett, Bray & Sleight, 1980; Hjalmarson, Herlitz, Malek, Ryden, Vedin *et al.*, 1981; The Norwegian Multicentre Trial, 1981) or are experimentally induced (Reimer, Rasmussen & Jennings, 1976; Nayler, Grau & Yepez, 1977; Kloner, Fishbein, Braunwald & Maroko, 1978; Nayler, Yepez, Fassold & Ferrari, 1978a; Carroll & Welman, 1979; Manning, Keogh, Shattock, Coltart & Hearse, 1981a; Burmeister, Reynolds & Lee, 1981). Why the administration of these agents affords protection is not entirely clear (Reimer *et al.*, 1976). A reduction in cardiac work due to β-adrenoceptor inhibition may be involved (Reimer *et al.*, 1976; Nayler *et al.*, 1977; Welman, 1979) particularly if catecholamines are released in response to oxygen deprivation (Wollenberger & Shahab, 1965; Wollenberger & Krause, 1968; Wollenberger, Krause & Heier, 1969; Rabinowitz, Kligerman & Parmley, 1975; Hirche, Franz, Bos, Bissing & Schramm, 1980; Holmgren, Abbahamsson, Almgreen & Erriksson, 1981). There is other evidence, however, which indicates that other properties of the β-adrenoceptor antagonists may also be involved. Thus Nayler *et al.* (1978a) found a protective effect for racemic propranolol 72 h after pretreatment with propranolol had been terminated, indicating that the protective effect of propranolol outlasts its β-adrenoceptor blocking activity. A similar finding has now been described for oxprenolol (Manning, Keogh, Coltart & Hearse, 1981b). In addition, both Sakai & Spieckermann (1975) and Nayler, Fassold & Yepez (1978b) found only a partial reduction of hypoxia-induced damage in hearts that had been depleted of catecholamines by reserpine pretreatment. Also 3-methylpropranol, an analogue of propranolol which lacks any β-

[1]Present address: Research Department, Pharmaceuticals Division, CIBA-GEIGY Ltd, CH-4002, Basle, Switzerland.

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adrenoceptor blocking activity (Ku & Lucchesi, 1978; Gross, Warltier & Hardman, 1978), is nearly as effective as propranolol in delaying or preventing the damage caused by prolonged episodes of ischaemia.

To investigate further the processes whereby β-adrenoceptor antagonists protect the myocardium against the deleterious effects of ischaemia and reperfusion, we have investigated the effect of chemical sympathectomy, using 6-hydroxydopamine (6-OHDA). 6-OHDA can be used to reduce the noradrenaline content of rabbits hearts by about 97% (Fozard, Kelly & Small, 1973; Jonsson & Sachs, 1975). Now, if the protective effect of β-adrenoceptor blockade is due simply to the interaction of the antagonists with the β-adrenoceptors, so that the positive inotropic and chronotropic effect of any released or circulating catecholamines is avoided, then theoretically, chemical sympathectomy should be equi-effective. The effectiveness of a particular protective regime can be assessed in several different ways. Altered rates of enzyme leakage can be monitored, ultrastructural damage quantitated, or the recovery of mechanical activity and mitochondrial function assessed. There are several reasons why we chose not to use enzyme leakage as a monitor of myocardial damage in this study. Firstly, it is possible that chemical sympathectomy may alter the leakage process. Secondly enzyme leakage is a late event. indicative of gross ultrastructural damage and the appearance of large membrane defects. Instead, because of the close association which has been established between the preservation of mitochondrial function and protection against ischaemic and reperfusion damage (Henry, Shuchleib, Davis, Weiss & Sobel, 1977; Henry, Shuchleib, Borda, Roberts, Williamson & Sobel, 1978; Nayler, Ferrari & Williams, 1980; Nayler, 1981a) we used mitochondrial function and Ca^{2+} content as our main marker. Mitochondrial function was assessed in terms of respiratory function, ATP producing activity and Ca^{2+} content (Nayler *et al.*, 1978a; 1980). In a second series of experiments the recovery of mechanical function, as indicated by peak developed tension, was also monitored.

Methods

Adult male New Zealand white rabbits weighing 2–2.5 kg were randomly divided into two groups. One group received an initial intravenous dose of 30 mg/kg 6-OHDA followed 20 h later by a further dose of 20 mg/kg and 4 h later by a final dose of 20 mg/kg. This schedule reduces the noradrenaline content of rabbit hearts by about 97% (Fozard *et al.*, 1973). 6-OHDA was always freshly dissolved in 0.7 ml ice cold sterile 0.9% w/v NaCl solution (saline) containing 1% ascorbic acid immediately before injection. It was injected as a 0.7 ml bolus.

The second group of rabbits (saline controls) received intravenous injections of 1% ascorbic acid in saline in the same dose regimen as the 6-OHDA-treated animals. Animals were killed 17–20 h after the third injection of either 6-OHDA or saline.

Perfusion

The rabbits were killed by cervical dislocation. The thorax was opened and the hearts rapidly excised and placed in heparinized (100 iu/100 ml) ice-cold Krebs Henseleit buffer previously equilibrated with 95% O_2 and 5% CO_2; 1 min later, and hence at a time when contractions had ceased, the aorta was cannulated and perfused at 37°C at a constant mean pressure of 60 mmHg (8.0 kPa) by the non-recirculating Langendorff perfusion technique (Langendorff, 1895). The perfusion buffer was Krebs Henseleit solution containing (mmol/l): NaCl 115.0, $NaHCO_3$ 25.0, KCl 4.0, KH_2PO_4 0.9, Mg_2SO_4 1.1, $CaCl_2$ 1.5 and glucose 11.0. The perfusate was gassed with 95% O_2 and 5% CO_2, giving a $Po_2 > 600$ mmHg (80 kPa). Perfusion pressure was monitored continuously, by means of a direct reading pressure gauge attached to a side arm of the inflow cannula. The pressure was maintained with a Watson-Marlow flow inducer (MK III, Marlow, England). Perfusion pressure was monitored continuously by means of a direct reading pressure gauge attached to a side arm of the inflow cannula. The output from the pressure gauge operated a negative-feed back circuit connected to the pump. In this way the pump output was automatically adjusted to provide a constant perfusion pressure. A small incision was made in the left atrium and the tissue around the pulmonary artery was carefully removed, to prevent any obstruction of the outflow tract.

Unless otherwise stated the hearts were paced, using suprathreshold rectangular pulses of 10 ms duration delivered from a Tektronix (Type 162) square valve generator assembly (Tektronix Inc., Portland, Oregon, USA) at a rate of 180/min. The stimuli were delivered via two platinum electrodes, one attached to the metal inflow cannula and the other to the ventricular apex.

Two separate groups of experiments were undertaken. In the initial series (Series I) tension generation was not monitored. In the second series (Series II) peak developed tension was monitored by means of a Narco Biosystems myograph (F-60) (Houston, Texas) attached via a nylon ligature to the apex of the left ventricle. The output from the myograph was displayed on a Narco Biosystems MK IV physio-

graph. At the start of each series II experiment, resting tension was adjusted until the developed tension reached its peak. This involved applying a resting tension of 3.1 ± 0.4 g (mean ± s.e., 36 expts).

Peak developed tension is defined as the difference between peak systolic and resting tension.

Experimental procedure

After 20–30 min aerobic perfusion (P_{O_2} > 600 mmHg), and irrespective of whether tension generation was being monitored, the hearts in both the control and 6-OHDA pretreated groups were randomly divided into three groups. In the first group aerobic perfusion was continued for a further 120 min. The second group was perfused aerobically for another 30 min and then made ischaemic by total cessation of the coronary flow for 90 min. The third group underwent 90 min total ischaemia followed by 30 min aerobic reperfusion at a constant mean pressure of 60 mmHg. The temperature of the hearts was continuously monitored by a miniature thermoprobe placed in the wall of the left ventricle and was maintained at 36–37°C throughout the entire experiment, irrespective of coronary flow. The temperature of the water jacket was automatically adjusted to correct for any change in left ventricular wall temperature. To prevent drying of the outer surface of the heart, a wad of cotton wool soaked in Krebs-Henseleit buffer was attached to the inner surface of the water jacket chamber. Preliminary studies confirmed that this procedure ensured that the chamber remained sufficiently moist to prevent the heart from becoming dehydrated. At the end of the experiments the hearts were dropped into ice cold buffer solution containing, unless otherwise stated: 0.18 mol/l KCl, 10.0 mmol/l disodium edetate (Na_2EDTA) and 5% bovine serum albumin (pH = 7.3–7.4) and the mitochondria isolated (see below). If the harvested mitochondria were to be assayed for Ca^{2+} the hearts were dropped into an ice-cold solution containing 0.12 mol/l KCl and 4 mmol/l Tris buffer, pH 7.2, without any Na_2 EDTA (Nayler *et al.*, 1980).

Isolation of mitochondria

(a) *for respiratory function studies* Left ventricular muscle was weighed, finely chopped and homogenized in the above EDTA-containing buffer solution to provide a final concentration of about 10 mg/g wet heart weight. This mince was then homogenized using an Ultra Turrex homogenizer operating at half speed for 5 s. The mitochondria were isolated by differential centrifugation, according to a modification of the method of Sordahl, McCollum, Wood & Schwartz (1973) as previously described (Nayler *et al.*, 1978a). The final pellet was suspended in a solution containing 0.12 mol/l KCl and 4 mmol/l Tris buffer pH 7.2, to give a protein concentration of about 15 mg/ml, measured by the method of Bradford (1976), and standardized against bovine serum albumin.

(b) *for Ca^{2+} content* The procedure used for isolating cardiac mitochondria so that they retain their endogenous Ca^{2+} has been described in detail elsewhere (Nayler *et al.*, 1980). Essentially it consists of homogenizing the heart in an EDTA-free, albumin-free medium (Peng, Kane, Murphy & Straub, 1977).

Measurement of mitochondrial respiration

The respiratory activity of the isolated mitochondria was measured at 25°C using a Gilson Oxygraph and a Clark or Rank oxygen electrode (Nayler *et al.*, 1978b; 1980). The incubation medium contained (mM): sucrose 250, Tris 10, glutamate 3.0, KH_2PO_4 3.0, EDTA 3.0 and ADP 0.5 (pH = 7.3–7.4); 0.75 mg mitochondrial protein was added per ml of reaction medium. The parameters used to assess mitochondrial oxidative phosphorylation were: (1) State QO_2, natoms oxygen used per mg mitochondrial protein per min in response to the addition of ADP. (2) State 4 QO_2, the basal rate of oxygen consumption in the absence of ADP (natoms oxygen used per mg protein per min). (3) Respiratory control index (RCI), State 3 QO_2/State 4 QO_2, is the ratio of oxygen consumed in the presence of ADP to that taken up after all of the ADP has been converted to ATP. It is an index of the tightness of coupling between oxygen consumption and phosphorylation. (4) ADP: O ratio, nmol of ADP used per natoms of oxygen consumed. This is an index of the efficiency of oxidative phosphorylation.

Duplicate estimations of these parameters were always made and an average value taken.

Measurement of mitochondrial ATP generation

ATP-generating capacity of mitochondria was measured at 25°C in the same reaction medium as described for the oxidative phosphorylation studies. Mitochondria were added to give final concentration of 0.37 mg mitochondrial protein per ml reaction medium and the reaction started by adding ADP 1.25 mM. At timed intervals after starting the reaction 200 µl samples were removed and added to ice cold 10% perchloric acid, to stop the reaction. Samples were centrifuged (200 *g* for 10 min; 0°C), the supernatant decanted, frozen and assayed for ATP by measuring the change in optical absorbance at 300 nm when NADP is reduced to NADPH (Nayler *et al.*, 1980).

Measurement of mitochondrial Ca^{2+}

Mitochondria that were assayed for Ca^{2+} were digested in HNO_3. La^{3+} (as $LaCl_3$) was added to suppress interference. The Ca^{2+} content of the digests was assayed by atomic absorption spectrometry, using a Varian AA-175 spectrometer as described elsewhere (Nayler *et al.*, 1980).

Reagents

All chemicals were of analytical reagent grade. 6-Hydroxydopamine HCl was obtained from Sigma Ltd, Poole, Dorset.

Statistical analysis

Results are expressed as mean ± s.e. of n experiments. Student's t test was used and $P = 0.05$ taken as the limit of significance.

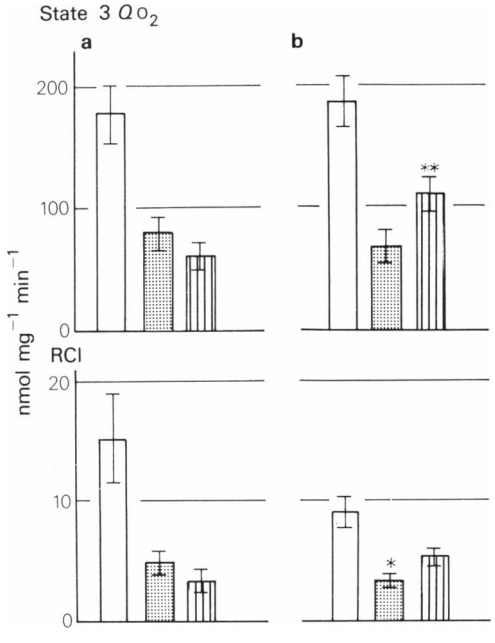

Figure 1 State 3 Q_{O_2} (nmol O_2 per mg mitochondrial protein and per min: nmol mg^{-1} min^{-1}) and respiratory control index (RCI) values of mitochondria extracted from hearts perfused aerobically (open columns), made ischaemic (stippled columns) or made ischaemic then reperfused (striped columns) (see text). Each value is mean of duplicate measurements from at least 6 separate experiments. Bars denote s.e.mean. Tests of significance were calculated by Student's t test and refer to the difference between the saline (a) and the 6-hydroxydopamine (6-OHDA)-treated groups (b) groups under the same experimental conditions.
$^*P < 0.05$; $^{**}P < 0.01$.
Note: these mitochondria were harvested from series I (see text) hearts. The equivalent data for series II hearts are listed in Table 4.

Results

Adequacy of catecholamine depletion

To ensure that the 6-OHDA regime we used had depleted the hearts of catecholamines, bolus doses of 500 μg tyramine were added to isolated spontaneously beating control and 6-OHDA-treated hearts which had been perfused aerobically for 30 min. When tyramine was added to the control (saline-treated) hearts the heart rate increased ($P < 0.001$) from 102 ± 14 to 168 ± 7 beats per min. In the 6-OHDA group the heart rate remained unchanged (98 ± 12 before tyramine, 103 ± 16 after tyramine). We deduced, therefore that our 6-OHDA pretreated hearts were catecholamine-depleted.

Mitochondrial function studies: Series I: experiments in which tension generation was not monitored

Effect of 6-OHDA pretreatment on mitochondrial function in aerobically perfused hearts Figure 1 and Tables 1–3 show that the 6-OHDA pretreatment regime that was used here had no effect on mitochondrial state 3 Q_{O_2}, RCI, ADP/O, Ca^{2+} content, ATP producing activity or yield, provided that the mitochondria were isolated from the aerobically perfused hearts. When added directly to freshly isolated mitochondria to provide a final concentration of up to 10^{-4} M, 6-OHDA had no effect on mitochondrial Ca^{2+}, RCI, state 3 Q_{O_2}, or their ability to rephosphorylate ADP to ATP.

Effect of ischaemia and reperfusion in the absence of 6-OHDA treatment

Oxidative phosphorylation Figure 1 shows that 90 min ischaemia reduced ($P < 0.001$) the ability of mitochondria from the saline-treated controls to use O_2 for state 3 respiration (state 3 Q_{O_2}). Reperfusion after 90 min ischaemia caused an exacerbation of this effect. Figure 1 also shows a similar trend for the RCI values with a significant reduction ($P < 0.001$) after 90 min ischaemia and a potentiation of this effect upon reperfusion. In agreement with our earlier findings this sequence of ischaemia and reperfusion had no effect (Table 1) on either the ADP:O ratio or the yield of mitochondrial protein (Nayler *et al.*, 1980).

Mitochondrial Ca^{2+} Table 2 shows that mitochondria harvested from untreated ischaemic hearts contained excess Ca^{2+} relative to the amount present after aerobic perfusion. Table 2 also shows that upon

Table 1 Mean values of the ADP:O ratio (nmol ADP used per natoms oxygen consumed) and the yield of mitochondrial protein (mg protein per g wet weight of ventricular muscle) of mitochondria isolated from hearts perfused at $Po_2 > 600$ mmHg for 120 min ('Aerobic'), made ischaemic for 90 min ('Ischaemic') or made ischaemic for 90 min then reperfused for 30 min ('Ischaemic-Reperfused')

Series I: Without tension record		*ADP:O ratio*	
Perfusion	*Aerobic*	*Ischaemic*	*Ischaemic-Reperfused*
Control (saline)	2.8±0.3 (5)	3.0±0.5 (6)	2.3±0.5 (5)
6-OHDA	2.6±0.2 (7)	2.7±0.3 (7)	2.5±0.3 (7)
Series II: With tension record			
Saline	2.6±0.2 (6)	2.7±0.3 (6)	2.2±0.4 (6)
6-OHDA	2.7±0.3 (6)	2.9±0.4 (6)	2.6±0.5 (6)
	Mitochondrial yield (mg protein per g wet heart wt)		
Series I: Without tension record			
Control (saline)	3.7±0.2 (10)	3.4±0.2 (10)	3.3±0.2 (10)
6-OHDA	3.5±0.3 (10)	3.5±0.4 (10)	3.1±0.4 (10)
Series II: With tension record			
Saline	3.5±0.4 (6)	3.3±0.2 (6)	3.2±0.4 (6)
6-OHDA	3.6±0.3 (6)	3.7±0.4 (6)	3.4±0.5 (6)

Number of separate experiments shown in parentheses. No significant difference (Student's *t* test) between saline and 6-hydroxydopamine (6-OHDA)-treated groups.

reperfusion there was a marked increase ($P < 0.001$) in mitochondrial Ca^{2+}.

ATP-production Relative to the aerobic control series, mitochondria from the ischaemic ($P < 0.01$) and ischaemic-reperfused hearts converted ADP to ATP (Table 3) relatively slowly ($P < 0.001$).

Effect of ischaemia and reperfusion after 6-OHDA treatment

Oxidative phosphorylation Figure 1 shows the effect of 6-OHDA pretreatment on the ischaemic and ischaemic-reperfusion-induced decline in mitochondrial oxidative phosphorylation. Although 6-OHDA pretreatment did not completely protect against the loss of mitochondrial function caused by the ischaemic episode it had some beneficial effect because mitochondria that were isolated from the reperfused hearts exhibited higher RCI ($P < 0.05$) and state 3 Qo_2 ($P < 0.01$) values than was found for the control saline-treated series (Figure 1).

Mitochondrial Ca^{2+} Table 2 shows a similar trend for Ca^{2+}, in that there was a smaller increase in mitochondrial Ca^{2+} upon reperfusion ($P < 0.01$) if the hearts from which the mitochondria were obtained came from 6-OHDA-treated, as opposed to saline-treated, rabbits.

Table 2 Effect of 6-hydroxydopamine (6-OHDA) pretreatment on the ischaemic and reperfusion-induced gain in mitochondrial Ca^{2+}

	Mitochondrial Ca^{2+} (nmol/mg protein)		
Perfusion	*Aerobic*	*Ischaemic*	*Ischaemic-Reperfused*
Series I: Without tension record			
Control (saline)	14.5±0.9	17.4±0.8	42.1±1.8
6-OHDA	14.2±0.8	16.3±0.6	30.4±0.6
Significance	NS	NS	$P < 0.01$
Series II: With tension record			
Control (saline)	14.2±1.1	19.6±0.6	49.8±2.3
6-OHDA	13.9±0.6	17.2±0.8	36.2±0.9
Significance	NS	NS	$P < 0.01$

Tests of significance relate to the protection due to 6-hydroxydopamine (6-OHDA) pretreatment against gain in Ca^{2+}. Conditions of perfusion as described in text. Each result is mean ± s.e. of 6 experiments. Aerobic refers to 120 min aerobic perfusion (see text); ischaemic refers to 90 min ischaemia at 37°C, and ischaemic-reperfused to 90 min ischaemia followed by 30 min reperfusion at 37°C.

Table 3 Effect of 6-hydroxydopamine (6-OHDA) pretreatment on the ischaemic and reperfusion-induced decline in mitochondrial ATP-producing activity

	ATP production (μmol ATP/mg protein)			
	Series I:		*Series II:*	
Duration of incubation (s)	30	60	30	60
Perfusion Aerobic				
Control	0.34±0.02	0.51±0.04	0.36±0.02	0.55±0.02
6-OHDA	0.37±0.06	0.57±0.06	0.39±0.04	0.59±0.06
Significance	NS	NS	NS	NS
90 min ischaemia				
Control	0.10±0.02	0.11±0.04	0.09±0.03	0.10±0.02
6-OHDA	0.12±0.03	0.13±0.06	0.10±0.02	0.12±0.05
Significance	NS	NS	NS	NS
90 min ischaemia-reperfusion				
Control	0.09±0.02	0.10±0.01	0.06±0.03	0.08±0.02
6-OHDA	0.13±0.02	0.14±0.03	0.10±0.02	0.11±0.03
Significance	$P<0.05$	$P<0.05$	$P<0.05$	$P<0.05$

Tests of significance relate to the significance of the difference due to 6-OHDA pretreatment. NS, not significant at level of $P=0.05$. Rates of ATP production were measured over periods of 30 and 60 s as indicated. Control refers to saline pretreatment.

ATP production Pretreatment with 6-OHDA failed (Table 3) to prevent 90 min ischaemia from severely impairing the mitochondrial ATP-producing activity. 6-OHDA pretreatment did, however, provide some protection ($P<0.05$) against the exacerbation of the loss of ATP-producing activity caused by reperfusion (Table 3).

Mitochondrial function studies: Series II: experiments in which tension generation was monitored

Effect of 6-OHDA pretreatment on the recovery of developed tension on reperfusion Although there was no significant difference between the peak tension developed by the control (saline-pretreated) (22.1±3.4 g, mean±s.e., 18 expts) and the 6-OHDA pretreated (21.9±4.2 g, mean±s.e., 18 expts) hearts before they were made ischaemic, upon reperfusion the control hearts recovered only 21% of their initial tension generating activity, whilst the 6-OHDA series recovered 38%. This difference was significant ($P<0.05$).

Control mitochondrial function studies

Attaching a transducer to the left ventricle, and applying a resting tension of 3.1±0.4 g (see above) imposes a work load on the heart. The presence of such a work load may have affected how well the heart and its mitochondria recovered from periods of ischaemia and reperfusion. Some additional experiments were therefore undertaken, in which mitochondrial oxidative phosphorylating activity, ATP generating capacity and Ca^{2+} content was determined using mitochondria harvested only from hearts which had been used for tension recordings. The results of these experiments are shown in Tables 1–4. Overall, the results closely resemble those already described for the series I experiments. Thus (Series II Table 1) under these conditions 6-OHDA pretreatment had no effect on either the ADP:O ratio or the mitochondrial yield, and it decreased (Table 2) but did not prevent the gain in mitochondrial Ca^{2+} that occurs during post-ischaemic reperfusion. Table 2 does show, however, that in the series II experiments the gain in Ca^{2+} exhibited by mitochondria from the ischaemic-reperfused hearts exceeded that ($P<0.05$) of the series I hearts, indicating that the imposition of the work load associated with the presence of the myograph had affected the capacity of the ischaemic myocardium to maintain homeostasis with respect to Ca^{2+}.

6-OHDA pretreament still exerted a mild protective effect on the mitochondrial ATP producing activity (Table 3), state 3 QO_2 (Table 4) and RCI (Table 4). There was no significant difference between the ATP producing activity (Table 3) and the RCI or state 3 QO_2 (Table 4 and Figure 1) of

Table 4 Effect of 6-hydroxydopamine (6-OHDA) pretreatment on state 3 Q_{O_2} and RCI of mitochondria prepared from the aerobically perfused, ischaemic and reperfused series II hearts

Perfusion	*Aerobic*	*Ischaemic*	*Ischaemic-Reperfused*
Q_{O_2} (nmol O_2 mg^{-1} min^{-1})			
Control	161 ± 22	72 ± 14	52 ± 16
6-OHDA	176 ± 34	76 ± 12	84 ± 17
Significance	NS	NS	$P < 0.05$
RCI			
Control	15.3 ± 3.6	3.6 ± 0.6	3.2 ± 0.2
6-OHDA	11.0 ± 3.2	4.3 ± 0.7	5.1 ± 0.6
Significance	NS	NS	$P < 0.05$

Each result is mean ± s.e. of 6 experiments. Tests of significance relate to the significance of the 6-OHDA treatment. Aerobic refers to 120 min aerobic perfusion (see text); ischaemic refers to 90 min ischaemia at 37°C, and ischaemic-reperfused to 90 min ischaemia followed by 30 min reperfusion, at 37°C. Control refers to saline pretreatment.

mitochondria harvested from the series I and series II hearts.

Discussion

The main aim of this study was to determine whether chemical sympathectomy with 6-OHDA protects cardiac mitochondria against the damage caused by a prolonged episode of normothermic global ischaemia, and reperfusion. Only partial protection was obtained (Figure 1, Tables 2, 3 and 4), limited mainly to protection against the exacerbation of damage during reperfusion. When compared with our previous results relating to the beneficial effect of pretreatment with either propranolol (Nayler *et al.*, 1980) or timolol (Nayler, 1981b) the protection obtained with 6-OHDA was not very impressive. Nevertheless the results are in agreement with the observation that reserpine pretreatment does little to protect the myocardium against the deleterious effects of hypoxia (Sakai & Spieckermann, 1975; Nayler *et al.*, 1978b).

Why catecholamine-depletion should be less protective than β-adrenoceptor blockade is puzzling. Isolated Langendorff perfused hearts do contain catecholamine and noradrenaline is released during an ischaemic episode (Nayler & Sturrick, unpublished data). There can be little doubt that the 6-OHDA pretreatment was effective because after it had been used, bolus doses of tyramine failed to elicit an increase in heart rate. Nor can we explain our failure to detect adequate protection after 6-OHDA treatment in terms of faulty mitochondrial preparations. The values obtained for State 3 Q_{O_2} and RCI agree with those found by others (Lindenmeyer, Sordahl & Schwartz, 1968; Schwartz, Sordahl, Entman, Allen, Reddy, Goldstein, Luchi & Wyborny, 1973; Lochner, Kotze & Gevers, 1976) using glutamate as the respiratory substrate (Peng *et al.*, 1977). The ADP:O ratios were also unaltered by ischaemia (Table 1), a finding which agrees with other results in the literature (Kane, Murphy, Pisset, de Soyza, Docherty & Straub, 1975; Peng *et al.*, 1977; Nayler *et al.*, 1980). Since the yield of mitochondria was unchanged (Table 1) we cannot explain our failure to detect adequate protection in terms of a selective recovery of mitochondria.

We are left with the problem of explaining why in the present study we obtained relatively little protection after catecholamine depletion whereas in our earlier studies we found pretreatment with propranolol to be effective (Nayler *et al.*, 1980). A possible explanation could be that the 6-OHDA regime we followed failed to deplete a particular store of catecholamines that is sensitive to oxygen deprivation. However, we find this to be an unsatisfactory explanation. Perhaps the use of mitochondrial function as an indicator of protection is unsatisfactory. This also seems to be an improbable explanation, because there are many studies which point towards a positive correlation between maintenance of mitochondrial function and myocardial preservation (Trump, Mergner, Won Kahng & Saladino, 1976; Henry, Schuchlieb, Davis, Weiss & Sobel, 1977; Weishaar & Bing, 1980; Nayler *et al.*, 1980). In any case our failure to obtain adequate protection by means of 6-OHDA pretreatment was not limited to the mitochondrial function studies, because the 6-OHDA-treated hearts that were made ischaemic and reperfused recovered only 38% of their initial tension-generating activity, which is less ($P < 0.002$) than that obtained after pretreatment with propranolol (56%) or timolol (63%) (Nayler *et al.*, 1980; Nayler, 1981b).

Possibly it is the interpretation of the results that is in error. In studies where we (Nayler *et al.*, 1980) and

others (Reimer *et al.*, 1973) have found the administration of certain β-adrenoceptor antagonists to be protective it has been tacitly assumed that it is the β-adrenoceptor blocking activity of these substances that is solely responsible for their protective effect. This may not necessarily be true because these compounds have other properties. For example some of them stabilize lysosomal membranes (Carroll & Welman, 1979; Welman, 1979), and some preferentially protect mitochondrial ultrastructure (Kloner *et al.*, 1978). In high concentrations some exert general membrane stabilizing and local anaesthetic properties. Possibly high tissue concentrations were achieved in the animals that were pretreated with β-adrenoceptor antagonists for several days before isolating their hearts and making them ischaemic (Nayler *et al.*, 1980; Manning *et al.*, 1981a), or hypoxic (Nayler *et al.*, 1978b). This could explain why under those circumstances the protective effect of administering a β-adrenoceptor antagonist extends beyond the duration of effective β-blockade (Nayler *et al.*, 1978a) and beyond the time when the plasma contains detectable levels of the antagonists (Manning *et al.*, 1981a). The recent paper of Arnim & Welman (1981) which showed that acutely administered doses of propranolol (3.4×10^{-7} M) that produce effective β-adrenoceptor blockade fail to prevent excessive creatine kinase release during postischaemic reperfusion lends support to the argument that the ability of certain β-adrenoceptor antagonists to protect the isolated myocardium against ischaemic and reperfusion-induced damage may involve other properties in addition to β-adrenoceptor blockade.

This does not imply that β-adrenoceptor blockade itself is not important. Indeed there is good evidence that it is protective (Parratt, Marshall & Ledingham, 1980; Manning *et al.*, 1981b). It may mean, however, that to account for the protective effect of substances like propranolol and timolol in terms of β-adrenoceptor blockade alone may be an oversimplification.

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