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Beneficial effect of amosulalol and phentolamine on post-hypoxic recovery of contractile force and energy metabolism in rabbit hearts

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1 The effects of phentolamine, an α-adrenoceptor blocking agent and amosulalol, an α_1 and β-adrenoceptor antagonist on hypoxia-induced impairment in cardiac function and metabolism were examined using the isolated heart Langendorff preparation of the rabbit.

2 Hypoxia induced cessation of cardiac contractile force, a rise in resting tension, a decrease in myocardial high-energy phosphates, an increase in tissue calcium content and the release of ATP metabolites from the heart. Subsequent reoxygenation resulted in little recovery of cardiac contractile force, and there were further increases in tissue calcium content and in the release of creatine kinase from the heart.

3 Treatment of hypoxic hearts with either 83 μM phentolamine or 45 μM amosulalol resulted in a suppression of the rise in resting tension, the tissue calcium accumulation and the release of creatine kinase and ATP metabolites during hypoxia. This treatment also elicited significant recovery of cardiac contractile force, restoration of myocardial high-energy phosphates, suppression of the release of creatine kinase and the accumulation of tissue calcium during reoxygenation. Both 83 μM phentolamine and 45 μM amosulalol exerted a significant prolongation of the effective refractory period of rabbit isolated atria.

4 Lower concentrations of phentolamine (16 μM) and amosulalol (9 μM), which are sufficient to exert an α-adrenoceptor blocking action, did not elicit an appreciable effect on the post-hypoxic recovery of cardiac contractile force.

5 These results suggest that phentolamine and amosulalol are capable of protecting the myocardium from hypoxia-induced derangements in cardiac function and metabolism. This effect is probably attributable to their membrane stabilizing effect, rather than to their α-adrenoceptor blocking action.

Introduction

Oxygen deficiency primarily induces functional and metabolic impairments in cardiac cells, which are believed to be dependent on the period and the severity of oxygen deficiency used (Hearse & Humphrey, 1975; Hearse *et al.*, 1977; Nayler *et al.*, 1979; Nayler, 1983; Murry *et al.*, 1986; Allen & Orchard, 1987). Several investigators have demonstrated a cardioprotective action of both calcium antagonists (Fleckenstein, 1971; Aschraf & Kahamathra, 1984) and β-adrenoceptor blocking agents in ischaemic hearts (Nayler *et al.*, 1978b; Wrangler *et al.* 1984). We have also shown that experimentally-induced hypoxic insults produce cardiac contractile failure, impairments in myocardial energy metabolism and changes in cell membrane permeability, which can be prevented by diltiazem, verapamil (Takeo *et al.*, 1988c) and coenzyme Q_{10} (Takeo *et al.*, 1987). α-Adrenoceptor blocking agents such as phentolamine and prazosin have been shown to be effective in protecting against post-ischaemic reperfusion-induced calcium accumulation in cat and rat hearts (Sharma *et al.*, 1983; Nayler *et al.*, 1985). This suggests that α- and/or β-adrenoceptor antagonists are beneficial for protection of the ischaemic or hypoxic myocardium, although the exact mechanism for the protection by these agents and the significance of the involvement of α- and β-adrenoceptors in the genesis and the development of the ischaemic injury are not fully understood. Amosulalol, 5-{1-hydroxy-2-(o-metho-

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xyphenoxy)ethyl)amino) ethyl}-2-methylbenzenesulphonamide hydrochloride, has been shown to elicit α_1- and β-adrenoceptor blocking effects (Takenaka *et al.*, 1982a; 1984; Honda *et al.*, 1986) but the effect of this agent on the ischaemic myocardium has not been studied in detail. The present study was undertaken to determine whether amosulalol has a protective action against hypoxia-induced alterations in cardiac function and metabolism. A representative α-adrenoceptor blocking agent, phentolamine, has also been examined in order to elucidate the possible contribution of α-adrenoceptors to this effect.

Methods

Perfusion of hearts

Male albino rabbits, weighing 1.8 to 2.2 kg, were anaesthetized with sodium pentobarbitone (35 mg kg^{-1}). Heparin (1000 u kg^{-1}) was also administered. Isolation and perfusion of hearts was carried out as described previously (Takeo *et al.*, 1987). After thoracotomy, the heart was quickly excised and chilled in a Krebs-Henseleit solution of the following composition (mmol l^{-1}): NaCl 120, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.25, $NaHCO_3$ 25, glucose 11. The heart was transferred to a non-recirculating Langendorff apparatus and perfused at 37°C with Krebs-Henseleit solution at a fixed flow rate of 16 ml min^{-1}. The perfusate was previously equilibrated with a gas mixture of 95% O_2 + 5% CO_2. The pH of the solution was 7.40 to 7.42. After a 10 min stabilizing period, the heart was paced at 180 beats min^{-1} under the stimulating conditions (duration, 1 ms; strength, 0.4V) by means of an electric stimulator (Nihon Kohden SEN-3201). The hearts were preloaded with a resting tension of 1.50 g. Cardiac contractile force was estimated by monitoring isometric tension development generated through a hook attached to the apex of the heart by means of a force displacement transducer (Nihon Kohden, TB-621T). Changes in resting tension and contractile force were displayed on an oscillographic thermal pen recorder (Nihon Kohden, WT-647G). After 25 min of perfusion (equilibration period), hearts were subjected to hypoxic perfusion with the buffer as described above, but with 11 mM mannitol replacing glucose to reduce anaerobic glycolysis rapidly. This perfusing solution was previously equilibrated with a gas mixture of 95% N_2 + 5% CO_2 (hypoxic conditions). After 25 min of hypoxic perfusion, the rabbit heart was perfused for 45 min with the Krebs-Henseleit solution containing glucose saturated with a gas mixture of 95% O_2 + 5% CO_2 (reoxygenated conditions). Po_2 values of the perfusing solutions for hypoxia and reoxygenation, when measured by means of a blood gas analyser (Denmark Radiometer, BMS-MK2), were 22 to 28 mmHg, and 620 to 645 mmHg, respectively. After perfusion under hypoxic or subsequent reoxygenated conditions, hearts were removed from the perfusion apparatus and analysed for cellular metabolites. Control hearts were perfused for 25 min under normoxic conditions in the absence of glucose (glucose-free normoxia), and then perfused for 45 min under normoxic conditions in the presence of glucose. Phentolamine (500 μg min^{-1}) and amosulalol (300 μg min^{-1}) were infused into the perfusate just before the aortic cannula at an infusion rate of 0.1 ml min^{-1} (83 μM phentolamine and 45 μM of amosulalol as the final concentration) during the hypoxic period, with an infusion pump (Termo STC-521). In a preliminary study, we examined the concentrations of the agents capable of eliciting a beneficial recovery of cardiac contractile force after post-hypoxic reoxygenation. Cardiac contractile force of the heart after 45 min reoxygenation under the present experimental conditions was 24% and 41% of the initial value when the heart had been treated with 9 and 18 μM amosulalol during the hypoxic perfusion, respectively, and 5% and 6% when the heart had been treated with 16 and 50 μM phentolamine (the mean values of two experiments). These recovery values for contractile force were much lower than those observed after treatment with 45 μM amosulalol or 83 μM phentolamine. These results motivated us to use 45 μM amosulalol or 83 μM phentolamine for the main study.

Myocardial high-energy phosphates

After perfusion of the hearts, a small apical portion of the left ventricle was rapidly frozen in liquid nitrogen. The frozen tissue was weighed, pulverized in a stainless steel tube with a stainless steel plunger under liquid nitrogen-cooling, and high-energy phosphate compounds extracted with 0.3 M $HClO_4$ and 0.25 mM ethylenediaminetetraacetate. The extract was neutralized with 2.5 M K_2CO_3, then centrifuged at 1,000 *g* for 20 min. The resulting supernatant fluid was employed as a sample for determination of adenosine triphosphate (ATP) and creatine phosphate (CP). Measurements of myocardial ATP content were performed according to the methods of Bücher (1947). For determination of myocardial CP content, the converting reaction of CP to ATP was employed according to the methods of Lowry & Passoneau (1972). After the conversion of CP to ATP, total ATP content of the myocardium in the reaction mixture was analysed according to the methods described above. Myocardial CP content

was calculated by subtracting the initial ATP content of the extract from the total ATP content. To determine the ratio of dry tissue weight to frozen tissue weight, a piece of frozen tissue was weighed. After drying at 120°C for 15 h, the tissue was weighed and the ratio was estimated. The mean value for dry tissue weight was 11.5 ± 0.7% of the frozen tissue.

Determination of calcium and water contents of the myocardium

After perfusion, about 100 mg of the left ventricle was sampled for determination of calcium and water contents of the tissue. The tissue was cut into four pieces, weighed and dried at 120°C for 15 h, and then tissue dry weight was estimated. Determination of tissue calcium content was performed according to the modified methods of Grochowski *et al.* (1976). The dried myocardium was digested for 4 h with 2.5 ml of 0.75 N HNO_3 at 25°C. After centrifugation of the digest for 10 min at 1,000 *g*, the supernatant fluid was diluted twice with 20 mM $LaCl_3$ – 0.1 N HCl. The calcium content was analysed in triplicate using an atomic absorption spectrometer (Shimazu AA-646, Kyoto).

Analysis of the perfusate eluted from the heart

During the perfusion, the perfusate eluted from the heart was collected at each minute of the perfusion. Ultraviolet absorbance of the perfusate, which is a rough estimate of the release of ATP metabolites from the perfused heart (Takeo *et al.*, 1988a) was monitored at 250 nm with a spectrophotometer (Hitachi 150-20). Changes in absorbance were expressed as the absorbance × 1000 g^{-1} wet weight tissue min^{-1}. All the perfusate from the heart during hypoxia and subsequent reoxygenation was also collected and analysed by means of a high-performance liquid chromatographic (h.p.l.c.) analyser (Hitachi 655), to determine net substances in the perfusate responsible for the absorbance at 250 nm. ATP metabolites were separated through a column of ODS-cellulose acetate, 4.6 mm in diameter and 15 cm in length (Cosmosil $5C_{18}$; Nakarai Chem. Co., Kyoto), by elution with 0.25 M $NH_4H_2PO_4$ and 5% CH_3CN at a flow rate of 1 ml min^{-1}. Absorbance of the eluate was monitored at 254 nm using a uv-detector (Uvilog 7). Retention times for ATP metabolites such as hypoxanthine, inosine and adenosine were 2.18, 2.85 and 6.34 min, respectively.

Creatine kinase activity of the perfusate

Creatine kinase (CK) activity of the perfusate eluted from the hypoxic and reoxygenated heart was measured according to the method of Bergmeyer *et al.* (1970).

Effect on maximal driving frequency of rabbit isolated atria

Male albino rabbits, weighing 1.8 to 2.2 kg were used and the hearts were set up in the Langendorff mode. The atria were rapidly dissected and suspended in a glass organ bath filled with a Krebs-Henseleit solution as described above. The medium was equilibrated with a gas mixture of 95% O_2 and 5% CO_2. An initial resting tension of 1 g was applied and the preparation was allowed to equilibrate for 30 min at 30°C. The isolated atria were electrically stimulated at 30°C by a 2 fold threshold stimulus with a duration of 1 ms. The maximal driving frequency was determined by identifying the frequency of the stimulus which the atria would no longer follow. After control maximal driving frequency was obtained, agents were applied to the organ bath. After 10 min equilibration, the maximal driving frequency was determined again in the presence of test drug. The maximal driving frequency was expressed as an effective refractory period.

Statistics

Results are expressed as the mean ± s.e.mean. Statistical signficance was calculated by use of Student's *t* test, paired *t* test or by analysis of variance followed by Dunnett's *t* test. Results with a probability of 5% or less were considered to be statistically significant ($P < 0.05$).

Results

Haemodynamic effects of the perfused heart

Rabbit isolated hearts were perfused for 25 min under hypoxic conditions in the presence and absence of either phentolamine or amosulalol, followed by 45 min reoxygenation in the absence of test drug. Changes in cardiac contractile force and resting tension are shown in Figure 1a and b, respectively. The initial developed tension, generated at an initial resting tension of 1.5 g, was 5.2 ± 0.4 g ($n = 59$). Contractile force of control hearts decreased to about 80% of the initial value after 25 min of normoxic perfusion. The contractile force of the heart markedly decreased immediately after the onset of hypoxia and reached nearly zero after 10 to 12 min of hypoxic perfusion. Reoxygenation of hearts pre-exposed to hypoxia for 25 min resulted in less than 10% recovery of the contractile force ($n = 6$). The rise in resting tension occurred about 5 min after the onset of hypoxia, and reached

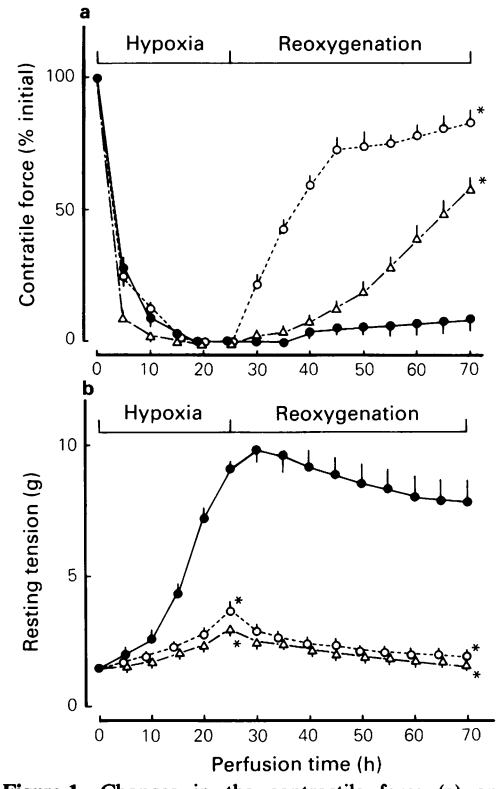

Figure 1 Changes in the contractile force (a) and resting tension (b) of the heart perfused for 25 min under hypoxic conditions in the absence (●) and presence of either phentolamine (△) or amosulalol (○), followed by 45 min of reoxygenation. Each value represents the mean of 6 experiments; vertical lines indicate s.e.mean. The results after 25 min of hypoxia and 45 min of reoxygenation were evaluated statistically. * Significantly different from the value for hearts without treatment with the agents ($P < 0.05$).

9.1 ± 0.3 g after 25 min of hypoxia ($n = 6$). A further small rise in resting tension was observed after 5 min of reoxygenation (9.8 ± 0.5 g; $n = 6$). The resting tension then gradually declined, but still remained at a level much higher than the initial level throughout reoxygenation.

When the heart was perfused under hypoxic conditions in the presence of either 45 μM amosulalol or 83 μM phentolamine, a significant suppression of the hypoxia-induced rise in resting tension was seen, although the hypoxia-induced decrease in the contractile force was not completely prevented by treatment with either agent. However, hearts which had received either 45 μM amosulalol or 83 μM phentolamine during hypoxia showed a marked recovery in contractile force after 45 min of reoxygenation; recovery of the contractile force of hearts treated with amosulalol (82.9 ± 4.4% of the initial value; $n = 6$) was greater than that with phentolamine (57.2 ± 3.8% of the initial value; $n = 6$). Perfusion of the heart for 25 min under normoxic conditions with glucose-free medium in the presence of either 45 μM amosulalol or 83 μM phentolamine, resulted in a decline in cardiac contractile force, which was followed by an appreciable recovery of the contractile force in the presence of glucose after a subsequent 45 min of normoxic perfusion (99.4 ± 2.4 or 69.2 ± 2.5% of the initial value, respectively, $n = 5$). There were no significant changes in resting tension of hearts perfused under normoxic conditions in the presence of either amosulalol or phentolamine.

High-energy phosphate content of the myocardium

After perfusion of the heart, myocardial high-energy phosphate content was measured and the results are shown in Figure 2. The initial values of ATP and CP contents were 24.6 ± 1.0 and 33.8 ± 3.0 μmol g^{-1} dry wt tissue, respectively ($n = 5$). When the heart was perfused for 25 min under normoxic conditions in the absence of glucose, myocardial ATP and CP contents decreased to 17.7 ± 1.2 and 15.7 ± 1.0 μmol g^{-1} dry wt tissue, respectively ($n = 4$). After reoxygenated perfusion with the medium containing glucose, these values tended to return toward the initial levels. The ATP content was almost completely restored, while the CP content recovered to approximately 85% of the initial level ($n = 4$). Perfusion of the heart for 25 min under hypoxic conditions resulted in a marked decrease in myocardial ATP and CP contents (7.4 ± 0.3 and 7.3 ± 0.3 μmol g^{-1} dry wt tissue, respectively; $n = 6$). Myocardial ATP and CP contents after 45 min of reoxygenation were 8.2 ± 0.2 and 13.6 ± 2.2 μmol g^{-1} dry wt tissue, respectively ($n = 6$), indicating little recovery of myocardial ATP and CP content upon reoxygenation. When the heart was subjected to hypoxic perfusion for 25 min in the presence of 45 μM amosulalol, myocardial ATP and CP contents (9.4 ± 0.4 and 9.7 ± 0.5 μmol g^{-1} dry wt tissue; $n = 6$) were slightly but significantly higher than those of hearts without the treatment. Tissue ATP content after 25 min of hypoxia in the presence of 83 μM phentolamine (11.6 ± 0.5 μmol g^{-1} dry wt tissue; $n = 6$) was higher than that not treated with phentolamine, whereas there were no significant differences in tissue CP contents of the hearts between the control and treated preparations. Treatment of hypoxic hearts with amosulalol significantly restored myocardial high-energy phosphates upon reoxygenation; myocardial ATP and CP after 45 min of reoxygenation were 22.6 ± 1.5 and 31.9 ± 1.1 μmol g^{-1} dry wt tissue, respectively

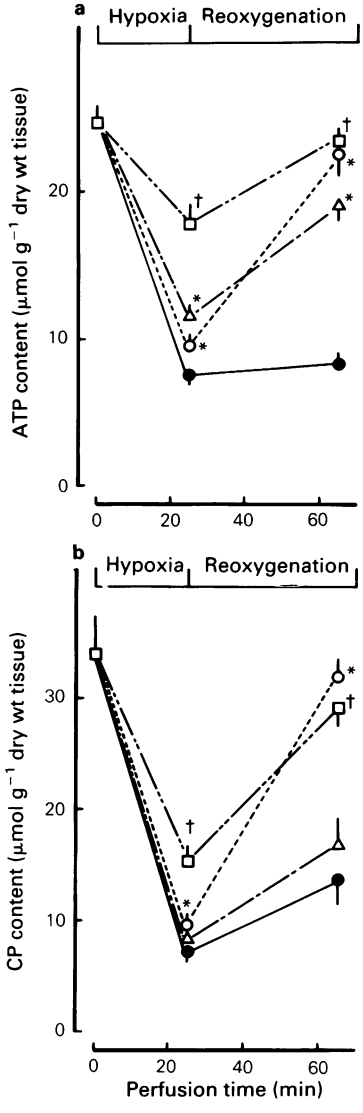

Figure 2 Myocardial ATP (a) and creatine phosphate (CP) (b) contents at 0, 25 min-hypoxia and 45 min-reoxygenation with or without (●) treatment of hypoxic hearts with either phentolamine (△) or amosulalol (○). Each value represents the mean of 4 to 6 experiments; vertical lines indicate s.e.mean. Statistical significance was evaluated between the hearts perfused under hypoxic and reoxygenated conditions in the absence and, the presence of the agents (*) or the hearts perfused under normoxic conditions (□) (†).

($n = 6$), which were almost the same as the control values. Similarly, treatment of hypoxic hearts with phentolamine significantly restored myocardial ATP and CP contents upon reoxygenation (80 and 50% of the initial value, respectively; $n = 6$). There was no significant difference in myocardial ATP and CP contents after 25 min and 65 min of normoxic perfusion between the treated and untreated preparations.

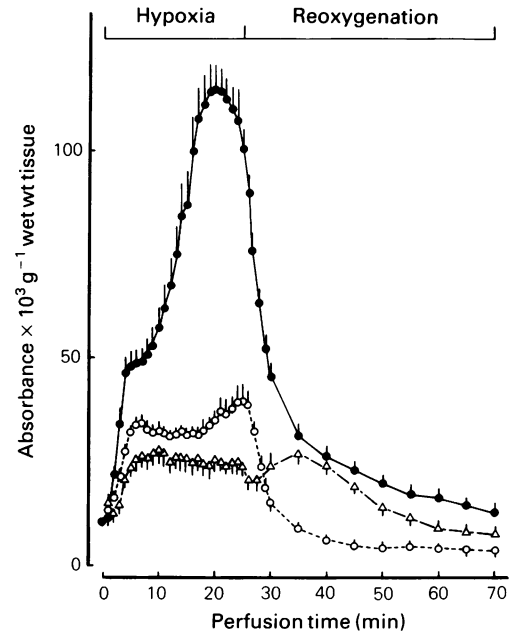

Figure 3 Changes in uv absorbance at 250 nm of the perfusate eluted from the perfused heart. (○) Represent values of the heart receiving amosulalol during hypoxia and (△) those of the heart receiving phentolamine. (●) Represent values obtained in the absence of treatment with the agents.

Changes in uv absorbance of the perfusate from perfused hearts

When the heart was exposed to hypoxia, an immediate increase in the absorbance at 250 nm of the perfusate eluted from the heart was seen, as shown in Figure 3. The absorbance was attenuated to a greater extent upon reoxygenation. Treatment of hypoxic hearts with either 83 μM phentolamine or 45 μM amosulalol markedly suppressed the hypoxia-induced increase in the absorbance of the perfusate.

H.p.l.c. analysis of the perfusate

To determine the substances in the perfusate responsible for an increase in the absorbance at 250 nm, samples of the perfusate during hypoxia and reoxygenation were analysed by h.p.l.c. The results are shown in Table 1. The control perfusate con-

Table 1 ATP metabolites released into the perfusate during hypoxia and subsequent reoxygenation

		Hypoxanthine	*Inosine*	*Adenosine*	*Total*
25 min-perfusion					
Normoxia without agents	(5)	ND	49 ± 4	ND	49 ± 4
Without treatment	(6)	221 ± 56*	1116 ± 214*	829 ± 185*	2166 ± 185*
Treatment with amosulalol	(6)	271 ± 49	18 ± 2†	13 ± 2†	302 ± 51†
Treatment with phentolamine	(6)	29 ± 9†	282 ± 55†	99 ± 25†	414 ± 88†
45 min-perfusion					
Normoxia without agents	(5)	ND	18 ± 10	ND	18 ± 10
Without treatment	(6)	362 ± 86*	1694 ± 302*	161 ± 2*	2217 ± 251*
Treatment with amosulalol	(6)	214 ± 90	16 ± 2†	2 ± 1†	232 ± 92†
Treatment with phentolamine	(6)	25 ± 10†	366 ± 40†	14 ± 7†	405 ± 45†

The heart was perfused for 25 min under hypoxic conditions in the presence and absence of phentolamine or amosulalol, followed by 45 min of reoxygenation. Values are expressed as nmol g^{-1} wet wt heart. Numbers in parentheses indicate number of experiments. ND indicates 'not detectable'. Each value represents the mean ± s.e.mean. * Significantly different from the value for the heart perfused under normoxic conditions ($P < 0.05$). † Significantly different from the value for the heart without treatment ($P < 0.05$).

tained mainly hypoxanthine and inosine and a minimal amount of adenosine. The sum of the three substances after 25 min of glucose-free normoxic and subsequently 45 min of normoxic perfusion was less than 70 nmol g^{-1} wet wt tissue. Release of hypoxanthine, inosine and adenosine was markedly increased under hypoxic conditions, which is consistent with previous results (Takeo & Sakanashi, 1983; Takew *et al.*, 1987; 1988a). The release of these compounds from the heart was also observed to a lesser extent during subsequent reoxygenation. Adenine nucleotides such as ATP, ADP and AMP or nicotinamide derivatives such as nicotine adenine dinucleotide (NAD), NAD reduced form (NADH), NAD phosphate (NADP) and NADPH were undetectable or negligible in the perfusate. Thus, the increase in absorbance was considered to be mainly due to the release of ATP metabolites from the perfused heart. Administration of 83 μM phentolamine during the hypoxic period significantly reduced the increase in ATP metabolites from the hypoxic and reoxygenated heart (81 and 82% reduction, respectively, $n = 6$). Suppression of the release of inosine and adenosine during hypoxia and reoxygenation was seen in hearts treated with amosulalol; more than 90% suppression was observed during the perfusion. No appreciable difference in ATP metabolite content of the perfusate was seen during normoxic perfusion in the presence of either phentolamine or amosulalol, as compared with that of the control heart.

Creatine kinase activity of the perfusate

Creatine kinase (CK) activity of the effluent from the perfused heart was measured. The results are shown in Figure 4. The initial value of the activity released from the perfused heart was 49 ± 5 μmol NADPH $min^{-1} g^{-1}$ wet wt tissue ($n = 59$). The CK activity of the perfusate after 25 min of glucose-free normoxic perfusion was slightly lower than the initial value. Hypoxia induced a significant increase in CK activity of the perfusate (151 ± 21 μmol NADPH $min^{-1} g^{-1}$ wet wt tissue after 25 min of hypoxia). Subsequent reoxygenation resulted in a further increase in CK release (1245 ± 383 μmol NADPH $min^{-1} g^{-1}$ wet wt tissue after 45 min of reoxygenation, $n = 6$). Perfusion of the heart under hypoxic conditions in the presence of either amosulalol or

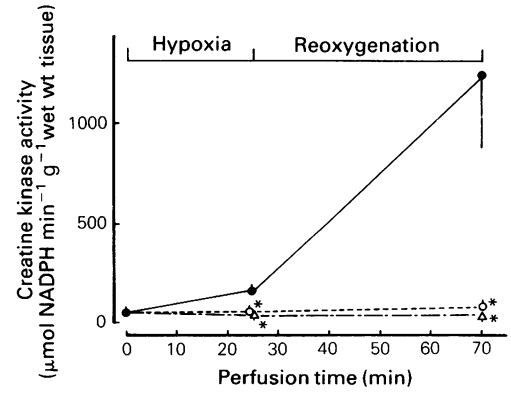

Figure 4 Creatine kinase activity of the perfusate at 0 min, 25 min-hypoxia and 45 min-reoxygenation in the absence (●) and presence of either phentolamine (△) or amosulalol (○). Each value represents the mean of 6 to 59 experiments; vertical lines indicate s.e.mean. * Significantly different from the value without treatment with the agents ($P < 0.05$).

phentolamine resulted in a marked suppression of the increase in CK release after 25 min of hypoxia (35 ± 10, and 13 ± 4 μmol NADPH $min^{-1} g^{-1}$ wet wt tissue, respectively, $n = 6$). Suppression of the release by these agents was also observed after 45 min of reoxygenation (amosulalol; 78 ± 24, and phentolamine; 81 ± 10 μmol NADPH $min^{-1} g^{-1}$ wet wt tissue, $n = 6$).

Calcium and water contents of the myocardium

The calcium and water contents in the left ventricle were measured after hypoxic and reoxygenated perfusion in the presence and absence of phentolamine and amosulalol. The results are shown in Figure 5. Calcium content of the heart perfused for 25 min under hypoxic conditions (3.7 ± 0.2 μmol g^{-1} dry wt tissue, $n = 6$) as well as for 45 min under subsequent reoxygenated conditions (4.4 ± 0.3 μmol g^{-1} dry wt tissue, $n = 6$) were significantly higher than the control (2.8 ± 0.3 μmol g^{-1} dry wt tissue, $n = 6$). The calcium content of the heart perfused under hypoxic conditions in the presence of either amosulalol or phentolamine (2.8 ± 0.1 and 2.6 ± 0.2 μmol g^{-1} dry wt tissue, respectively, $n = 6$) were significantly lower than those in the corresponding hearts in the absence of these agents. There was no significant difference in water content in the left ventricle, regardless of the conditions employed. Furthermore, under normoxic conditions, neither phentolamine nor amosulalol altered the calcium or water content of the perfused heart; for example, myocardial calcium content after 45 min of normoxic perfusion was 2.8 ± 0.2, and 2.9 ± 0.2 μmol g^{-1} dry wt tissue ($n = 5$), when hearts had been treated during 20 min glucose-free, normoxic perfusion with either phentolamine or amosulalol, respectively.

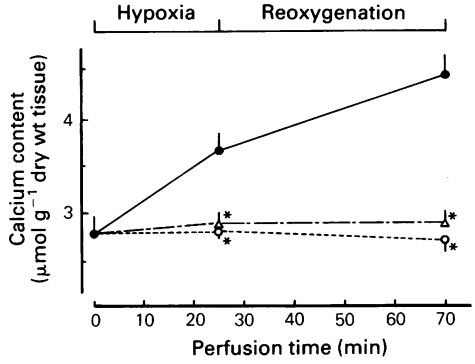

Figure 5 Myocardial calcium content at 0 min, 25 min-hypoxia and 45 min-reoxygenation with or without (●) treatment of hypoxic hearts with either phentolamine (△) or amosulalol (○). Each value represents the mean of 5 to 6 experiments; vertical lines indicate s.e.mean. * Significantly different from the value without the treatment ($P < 0.05$).

Maximal driving frequency of rabbit isolated atria

The maximal driving frequency was determined in the presence and absence of different concentrations of either phentolamine or amosulalol. The results are shown in Table 2. Amosulalol (45 μM) and phentolamine (83 μM) significantly prolonged the effective refractory period by 30 to 40%. In contrast, 30 μM or lower concentrations of phentolamine or less than 30 μM amosulalol did not elicit significant prolongation of the effective refractory period of the isolated atria.

Discussion

In the present study, rabbit isolated hearts were perfused under hypoxic and reoxygenated conditions. Under these conditions, we observed hypoxia-induced derangements in cardiac function such as cardiac contractile depression and rise in resting tension. It should be emphasized that the contractile force and the resting tension recovered little during subsequent reoxygenation. The hypoxic insults in the present study also induced changes in cardiac metabolism. Myocardial high-energy phosphates such as ATP and CP were significantly decreased to 30% of the initial value after 25 min of hypoxia, and

Table 2 Effects of phentolamine and amosulalol on the effective refractory period in rabbit isolated atria

		Control (ms)	*Effective refractory period* (% control) 1 μM	10 μM	30 μM	45 μM	83 μM
Phentolamine	(4)	158 ± 8	102 ± 3	109 ± 5	121 ± 12		139 ± 12*
Amosulalol	(5)	154 ± 8	100 ± 0	104 ± 2	116 ± 2*	133 ± 6*	

Effective refractory period was determined by measuring the maximal driving frequency of rabbit isolated atria. Effect of effective refractory period was expressed as % control. The initial resting sinus rate of the isolated atria used in the experiment for phentolamine and amosulalol was 115 ± 5 and 103 ± 6 beats min^{-1}, respectively. Numbers in parentheses indicate numbers of experiment.

* Significantly different from the control ($P < 0.05$).

were little restored after 45 min of reoxygenation. A significant accumulation of tissue calcium and release of CK from hearts was observed during hypoxic perfusion. These changes were further enhanced when the heart was reoxygenated. A release of ATP metabolites such as adenosine, inosine and hypoxanthine was also observed during hypoxic perfusion. These results are consistent with those described elsewhere (Hearse *et al.*, 1973; Schrader *et al.*, 1977; Nayler *et al.*, 1978a; Vary *et al.*, 1979; Poole-Wilson, 1980), suggesting that the experimental conditions are sufficient to produce oxygen-deficiency in most myocardial cells.

Brief periods of ischaemia are known to cause prolonged cardiac contractile dysfunction, so-called 'stunned myocardium' (Braunwald & Kloner, 1982). In a previous study (Nakahara & Takeo, 1986), it was observed that cardiac mitochondria were swollen or disrupted and the necrotic bands of myofibrils were clearly formed under the conditions similar to the hypoxic insults used in the present study. The present study also showed a release of CK from the perfused heart and an accumulation of tissue calcium during hypoxia and subsequent reoxygenation. According to Braunwald & Kloner (1982), a release of CK from the heart is a marker of irreversible damage in the myocardial cells. Furthermore, the perfusate in the present study does not contain humoral factors, hormones or nutrients except glucose, so that appreciable recovery in cardiac function and metabolism would not be expected to occur as long as myocardial cells are substantially damaged, even when oxygen is replenished. In fact, we observed in a preliminary study, that cardiac contractile force after 8 h of reoxygenation under the present experimental conditions was less than 10% of the initial value, which is similar to the contractile force after 45 min of reoxygenation. Thus, hypoxic conditions employed in the present study seem to be different from those producing stunned myocardium, and would seem to be more severe.

Treatment of hearts with a high concentration of either phentolamine (83 μM) or amosulalol (45 μM) during the hypoxic perfusion significantly suppressed the hypoxia-induced rise in resting tension, and release of CK and ATP metabolites from the heart, while hypoxia-induced decreases in cardiac contractile force and myocardial high-energy phosphates were influenced to a lesser degree. A marked effect of these agents was observed in the recovery of the contractile force upon reoxygenation; the contractile force of hearts treated with either amosulalol or phentolamine was 83 or 57% of the initial value, respectively. The recovery of the contractile force was found to be associated with the restoration of myocardial high-energy phosphates. Several investigators have demonstrated a close relationship between myocardial high-energy phosphates and recovery of cardiac contractile force after oxygen deficiency (Jennings *et al.*, 1978; Reibel & Rovetto, 1978; Hearse, 1979; Vary *et al.*, 1979; Takeo & Sakanashi, 1983). This is in agreement with our findings. Treatment of hypoxic hearts with amosulalol or phentolamine also prevented hypoxia- and reoxygenation-induced tissue calcium accumulation and reoxygenation-induced release of CK from the heart. These results indicate that treatment with these agents is capable of recovering cardiac function and metabolism after hypoxia. Sharma *et al.* (1983) have shown that treatment of anaesthetized cats with α-adrenoceptor blocking agents, 5 mg kg^{-1} phentolamine or 100 μg kg^{-1} prazosin, 2 min before reperfusion suppressed ischaemia/reperfusion-induced calcium overload. Also, Nayler *et al.* (1985) have demonstrated that pretreatment of the rat perfused heart with 1 μM prazosin before total ischaemia suppressed a gain in calcium caused by post-ischaemic reperfusion. These and the present findings suggest that some agents which possess α-adrenoceptor blocking actions are capable of protecting the myocardium from ischaemia-induced impairment of cardiac cells.

The protective action of α-adrenoceptor antagonists described above and phentolamine and amosulalol in the present study required the presence of high concentrations. In contrast, α-adrenoceptor blocking effects of phentolamine and amosulalol have been demonstrated at much lower concentrations of 0.1 to 1 μM (Shoji, 1981; Takenaka *et al.*, 1982b). If suppression of hypoxia-induced accumulation of tissue calcium was due to antagonism of α-adrenoceptors, treatment with lower concentrations might be expected to exert similar effects. We did not observe an appreciable effect of these agents on the hypoxic myocardium when lower concentrations of either amosulalol (9 μM) or phentolamine (16 μM) were used, suggesting that the protective effect seems not to be mediated through an α-adrenoceptor blocking action. This is supported by the postulation of Nayler *et al.* (1985) that prazosin-induced protection of the myocardium from ischaemic injury is due to a non-specific effect, and not antagonisms of α-adrenoceptors.

Amosulalol has been shown to exert selective α_1-adrenoceptor and non-selective β-adrenoceptor blockade with an α_1/β_1 ratio of one to one (Honda *et al.*, 1986). Its antagonistic potency at α_1 and β_1-adrenoceptors was respectively 4 times higher than phentolamine and 3 times less than propranolol (Takenaka *et al.*, 1982a). Since the results in the present study substantially failed to demonstrate an involvement of α-adrenoceptor blocking action in the beneficial recovery of post-hypoxic recovery of

cardiac function and metabolism, a β-adrenoceptor blocking action might be another possible mechanism in the case of amosulalol.

Apart from mechanisms relating to adrenoceptors, our results suggest several possible mechanisms underlying the action of amosulalol and phentolamine on hypoxic hearts. First, an energy sparing effect should be considered, like that attributed to the cardioprotective action of calcium antagonists (Fleckenstein, 1971). It should be noted that treatment with either amosulalol or phentolamine slightly but significantly preserved myocardial high-energy phosphates after 25 min of hypoxia. If this does play an important role in the recovery of cardiac contractile force, then an energy sparing effect may be relevant.

Secondly, preservation of ATP metabolites during hypoxic perfusion should be considered (Goldhaber *et al.*, 1982; Harmsen *et al.*, 1984). We have shown in a previous study that the release of ATP metabolites during hypoxic perfusion is related to the recovery of cardiac mechanical function (Takeo *et al.*, 1988a); that is, the greater the release of ATP metabolites during hypoxic perfusion, the lesser the recovery of cardiac contractile force during subsequent reoxygenation. Furthermore, in another set of experiments, we demonstrated that treatment with exogenous ATP metabolites such as adenosine, inosine and hypoxanthine during hypoxic perfusion suppresses hypoxia-induced derangements in cardiac function and metabolism, and facilitates the cardiac contractile force on replenishment of oxygen (Takeo *et al.*, 1988b). In the present study, treatment of hypoxic hearts with either amosulalol or phentolamine significantly suppressed the release of ATP metabolites from the heart during hypoxia and subsequent reoxygenation. This implies that ATP metabolites are preserved by the treatment with these agents, so that ATP synthesis is enhanced during subsequent reoxygenation.

Thirdly, protection of cardiac cells from hypoxia-induced increases in the cell membrane permeability should be considered. In the present study, treatment with either amosulalol or phentolamine during hypoxic perfusion significantly suppressed the release of CK from the perfused heart. The release of this enzyme is accepted to be a marker of an increase in cell membrane permeability (Hearse *et al.*, 1973; Hearse & Humphrey, 1975, Jennings, 1976; Ganote & Kaltenbach, 1979). Furthermore, we also observed a significant suppression in the accumulation of tissue calcium on treatment with these agents. It has been postulated that hypoxia-induced accumulation of myocardial calcium is caused by changes in cell membrane permeability (Poole-Wilson, 1980; Nayler, 1983; Nayler *et al.*, 1984). A similar conclusion was reached by Jarmakani *et al.* (1979) and Jennings *et al.* (1985, 1986). Thus, it is likely that treatment with these agents during hypoxic perfusion protects the myocardial cell membrane from hypoxia-induced increases in cell membrane permeability.

Another possible mechanism responsible for the cardioprotection of amosulalol and phentolamine may be a membrane stabilizing action. It is conceivable that this effect is related to the protective action of these agents against hypoxia- and reoxygenation-induced changes in cell membrane permeability, although, so far, there is no evidence that these two factors are correlated. In the present study, we have determined whether phentolamine and amosulalol are able to exert a membrane stabilizing action in concentrations used in the Langendorff preparation. For this purpose, effects of these agents on the maximal driving frequency of rabbit isolated atria were examined, since this action is believed to be a convenient indicator of the membrane stabilizing action of drugs (Benfey & Varma, 1966). It was found that both 83 μM phentolamine and 45 μM amosulalol produced a significant prolongation of the effective refractory period in rabbit isolated atria, suggesting a membrane stabilizing action of these agents at concentrations which elicited a beneficial effect on post-hypoxic recovery of cardiac function and metabolism. It should be mentioned that lower concentrations of these agents, such as 9 μM amosulalol and 16 μM phentolamine, which did not exert an appreciable effect on the post-hypoxic recovery of cardiac function and metabolism, appeared to produce little effect on the effective refractory period. Furthermore, phentolamine has been shown to elicit an inhibition of $\dot{V}_{max}$ of the action potential in rat ventricular muscle at concentrations ranging from 1 to 20 μM (Northover, 1983). These findings suggest that the beneficial effect of amosulalol and phentolamine observed in the present study is, at least in part, attributable to their membrane stabilizing action, rather than to antagonism of α-adrenoceptors.

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