0022-3565/91/2581-0094$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 258, No. 1
Printed in U.S.A.

Beneficial Effects of Yohimbine on Posthypoxic Recovery of Cardiac Function and Myocardial Metabolism in Isolated Perfused Rabbit Hearts[1]

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Accepted for publication April 1, 1991

ABSTRACT

The present study was undertaken to elucidate the possible actions of yohimbine on cardiac function and metabolism in the hypoxic and subsequently reoxygenated myocardium. For this purpose, rabbit hearts were perfused for 20 min under hypoxic conditions, followed by 45 min reoxygenated perfusion, and their functional and metabolic alterations with and without yohimbine treatment were examined. Hypoxia induced cessation of cardiac contractile force, rise in resting tension and depletion of tissue high-energy phosphates, which were poorly recovered by subsequent reoxygenation. Hypoxia also induced release of creatine kinase and ATP metabolites from perfused hearts and increases in tissue calcium and sodium contents, which were further enhanced upon subsequent reoxygenation. When hypoxic hearts were treated with 3 to 30 μM yohimbine, several beneficial effects were observed in a concentration-dependent manner. This included enhancement of posthypoxic recovery of contractile function and suppression of the hypoxia- and reoxygenation-induced rise in resting tension. Hypoxia/reoxygenation-induced release of ATP metabolites was inhibited and restoration of myocardial high-energy phosphates enhanced. Inhibition of reoxygenation-induced rise in tissue calcium and sodium and creatine kinase release were also noted. The findings suggest that suppression of transmembrane flux of ions, substrates and enzymes during hypoxia/reoxygenation plays a role in the posthypoxic functional and metabolic recovery. Yohimbine (3–30 μM) significantly depressed the maximal stimulus frequency the left atria could follow. These results suggest a close relationship between depression in the maximal driving frequency of atria and enhancement of the posthypoxic contractile and metabolic recovery of perfused hearts. The concentration of yohimbine which exhibits *alpha*-2 adrenoceptor blocking action has been reported to be lower than that required in this study. Thus, the present study suggests contribution of nonreceptor-mediated action of yohimbine to the improvement of cardiac function and metabolism of hypoxic and reoxygenated hearts.

Yohimbine, 17α-hydroxyyohimban-16α-carboxylic acid methylester, is an indolealkylamine alkaloid found in the bark of the *Pausinystalia yohimbe* tree or the root of *Rauwolfia*. The agent has many pharmacological actions, including modulation of catecholamine release in central and peripheral tissues. It also blocks or reverses catecholamine- or sympathomimetic drug-induced contraction of vas deferens and gastrointestinal tract, platelet aggregation, lipolysis in adipose tissue and suppression of insulin release (see review of Goldberg and Robinson, 1983). Because the agent exerts its action mostly through suppression of *alpha* adrenoceptor stimulation and because the mode of action and the radioligand binding activity of yohimbine are different from those of *alpha*-1 adrenoceptor and *beta* adrenoceptor blocking agents, yohimbine is now classified as an *alpha*-2 adrenoceptor blocking agent. In particular, yohimbine is considered to increase the release of catecholamines from presynaptic nerve terminals.

Several cardiovascular effects of yohimbine have been demonstrated, such as increases in blood pressure and heart rate in conscious animals and humans (Holmberg and Gershow, 1961; Ingram, 1962; Gomes *et al.*, 1980; Rockhold and Gross, 1981). In contrast, little information is available on the direct effects of yohimbine on cardiac function and myocardial metabolism under normal or oxygen-deficient conditions.

In previous studies from our laboratory, we have shown that *alpha* adrenoceptor blocking agents, such as phentolamine, bunazosin, YM-12617 and amosulalol (Takeo *et al.*, 1989; Tanonaka *et al.*, 1989a,b), and *beta* blocking agents such as propranolol and acebutolol (Takeo *et al.*, 1990), enhance posthypoxic recovery of contractile and metabolic function of isolated perfused rabbit hearts. Because these agents possess specific pharmacological properties of their own and the concentrations which exerted the beneficial effects were higher than those required for *alpha* or *beta* adrenoceptor blocking action, we

Received for publication November 29, 1990.
[1] This work was supported in part by a grant from The Science Research Promotion Fund from Japan Private School Promotion Foundation.

ABBREVIATION: CP, creatine phosphate.

concluded that the beneficial effects of these agents might not be attributed to either *alpha* blocking or *beta* blocking action. Consequently, we postulated another mechanism for the observed beneficial effects of these agents, that is, a possible role of suppression of transmembrane flux of substrates, ions and enzymes in this mechanism. Furthermore, we also showed that the beneficial effects of pretreatment with either propranolol or acebutolol during hypoxia were related to the effects of these agents on the maximal stimulus frequency left atrium could follow (the maximal driving frequency) (Takeo *et al.*, 1990). The effects on the maximal driving frequency are considered to be related to those on the refractory period of heart muscle (Vaughan Williams and Szekeres, 1961; Levy and Richards, 1965; Basil *et al.*, 1973). Moreover, prolongation of the effective refractory period may represent, quinidine-like, antiarrhythmic, local anesthetic or so-called "membrane stabilizing" activity (Dawes, 1946; Smith, 1982). This suggests an important role for quinidine-like action in the beneficial posthypoxic recovery of cardiac contractile and metabolic function.

Yohimbine has been demonstrated to exert local anesthetic effects in sciatic nerves and skeletal muscles at relatively higher concentrations (Shaw *et al.*, 1955; Simon, 1955), unrelated to its *alpha*-2 adrenoceptor blocking action. Thus, if the ability of drugs to protect against posthypoxic contractile and metabolic failure is strongly related to its ability to alter the maximal driving frequency, yohimbine, at a concentration which exhibits local anesthetic or quinidine-like action, may enhance recovery of cardiac function and metabolism after a period of hypoxia. The present study was undertaken to determine this possibility.

Methods

Effects on maximal driving frequency. Male Hartley guinea pigs, weighing 230 to 280 g, were used in this study on the maximal driving frequency of left atria. The animals were stunned by a blow on the head and their hearts were rapidly isolated and immersed in the oxygenated Krebs-Henseleit solution of the following composition (mM): NaCl 120, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11. The atria were dissected in the oxygenated buffer and suspended in a glass organ bath filled with buffer as above. The buffer was equilibrated with a gas mixture of 95% O_2 + 5% CO_2. After loading an initial resting tension of 0.50 g, the preparation was allowed to equilibrate for 30 min at 30°C. The left atria were paced at 200 beats/min during the initial phase with a stimulus of twice threshold and a duration of 1 msec by means of a square wave stimulator (Nihonkohden SEN-7203, Tokyo, Japan), and then stimulated at an increasing frequency. The maximal driving frequency was the stimulus frequency which the atria could not follow. This procedure was described previously (Takeo *et al.*, 1990).

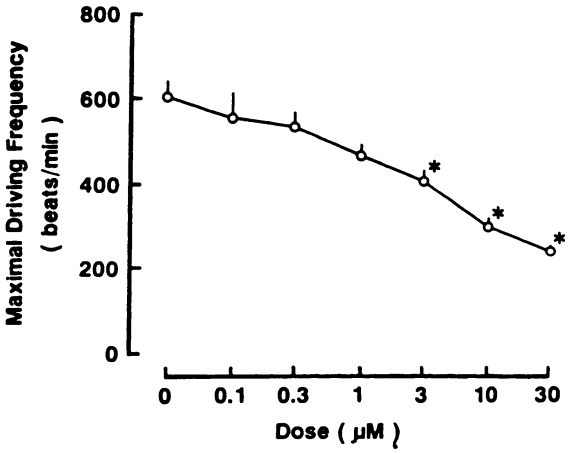

Fig. 1. The maximal driving frequency of the guinea pig left atria in the presence and absence (control) of various concentrations of yohimbine ranging from 0.1 to 30 μM. Values represent the mean ± S.E.M. of six experiments. Statistical significance was estimated using Dunnett's *t* test after analysis of variance. *Significantly different from control ($P < .05$).

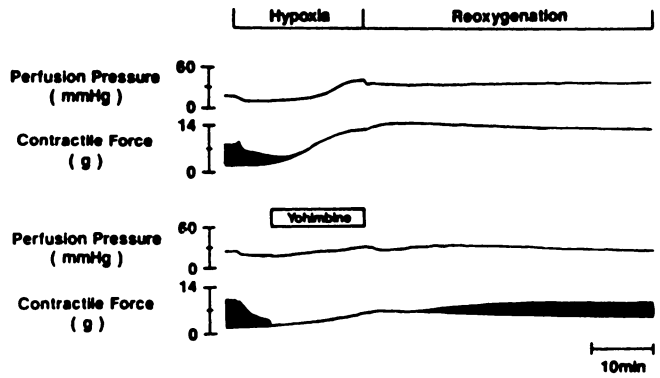

Fig. 2. Typical tracings of contractile force, resting tension and perfusion pressure of isolated rabbit hearts perfused under 20 min of hypoxic conditions and subsequent 45 min of reoxygenated conditions without (*upper panel*) and with 30 μM yohimbine treatment (*lower panel*). Administration of yohimbine was commenced at 5 min of hypoxic perfusion and terminated at 20 min of hypoxic perfusion.

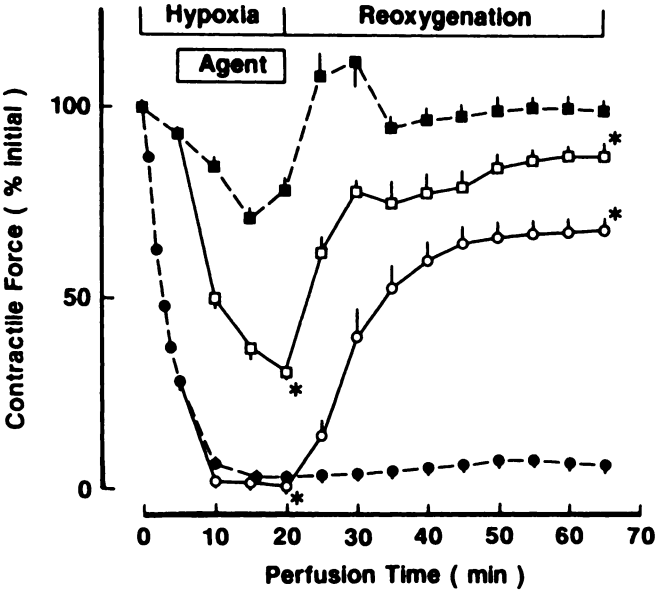

Fig. 3. Time course of changes in cardiac contractile force of isolated rabbit hearts subjected to hypoxia/reoxygenation with (○) and without 30 μM yohimbine treatment (●), and glucose-free normoxia/normoxic reperfusion with (□) and without 30 μM yohimbine treatment (■). Values represent the mean ± S.E.M. of 11 to 16 experiments for hypoxic or glucose-free normoxic hearts and six to 10 experiments for reoxygenated or normoxic reperfused hearts. Statistical significance was calculated using values of the groups at 20 min of perfusion and subsequent 45 min of perfusion with and without 30 μM yohimbine treatment. *Significantly different from the value without yohimbine treatment ($P < .05$).

Perfusion of rabbit hearts. Perfusion of rabbit hearts in the present study was similar to that in the previous study (Takeo *et al.*, 1989). In brief, male Japanese white rabbits, weighing 1.6 to 1.8 kg, were anesthetized i.v. with 35 mg/kg of sodium pentobarbital. Heparin (1000 U/kg) was administered simultaneously. After thoracotomy, rabbit hearts were isolated and perfused at 37°C with a constant flow rate of 16 ml/min with the Krebs-Henseleit solution of the following composition (mM): NaCl 120, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.25,

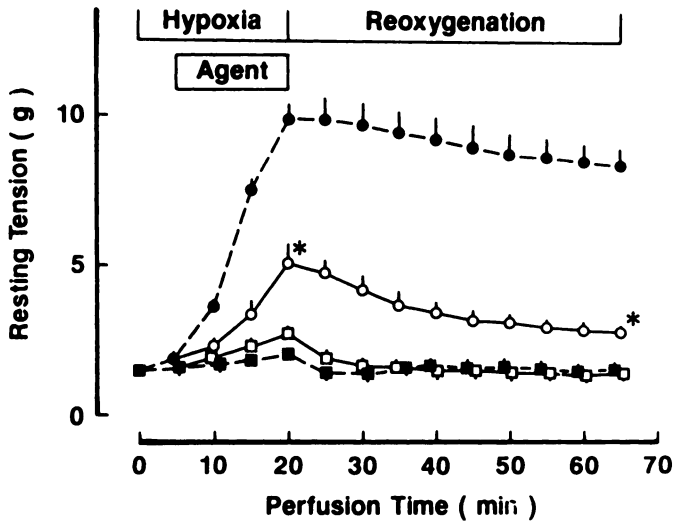

Fig. 4. Time course of changes in resting tension of isolated rabbit hearts subjected to hypoxia/reoxygenation with (○) and without 30 μM yohimbine treatment (●) and glucose-free normoxia/normoxic reperfusion with (□) and without 30 μM yohimbine treatment (■). Values represent the mean ± S.E.M. of 11 to 16 experiments for hypoxic or glucose-free normoxic hearts and six to 10 for reoxygenated or normoxic reperfused hearts. Statistical significance was calculated using values of the groups at 20 min of perfusion and subsequent 45-min of perfusion with and without 30 μM yohimbine treatment. *Significantly different from the value without yohimbine treatment ($P < .05$).

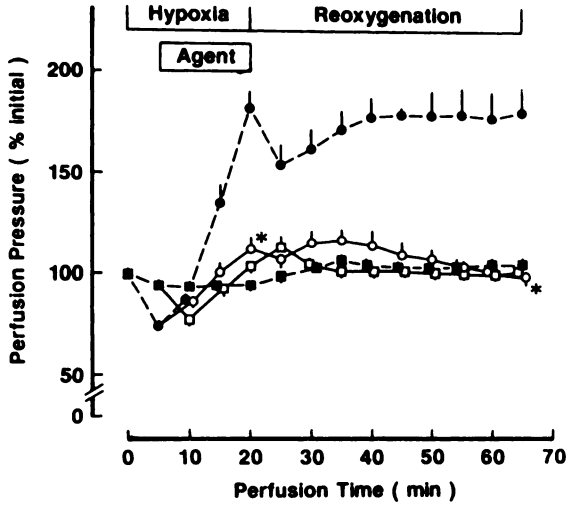

Fig. 5. Time course of changes in perfusion pressure (% initial) of isolated rabbit hearts subjected to hypoxia/reoxygenation with (○) and without yohimbine treatment (●) and glucose-free normoxia/normoxic reperfusion with (□) and without 30 μM yohimbine treatment (■). Values represent the mean ± S.E.M. of 11 to 16 experiments for hypoxic or glucose-free normoxic hearts and six to 10 experiments for reoxygenated or normoxic reperfused hearts. Statistical significance was calculated using values of the groups at 20 min of perfusion and subsequent 45-min of perfusion with and without yohimbine treatment. *Significantly different from the value without yohimbine treatment ($P < .05$).

$NaHCO_3$ 25 and glucose 11. The perfusing medium was equilibrated with a gas mixture of 95% O_2 + 5% CO_2. The heart was preloaded with an initial resting tension of 1.5 g and paced at 180 beats/min. Perfusion pressure was monitored through a branch of an aortic cannula mounted on the heart, by means of an electronic manometer (Nihonkohden, TP-101T, Tokyo, Japan). Cardiac contractile force was estimated by monitoring isometric tension development generated with the initial resting tension, through a hook attached to the apex of the heart by means of a force-displacement transducer (Nihonkohden, TB-612T,

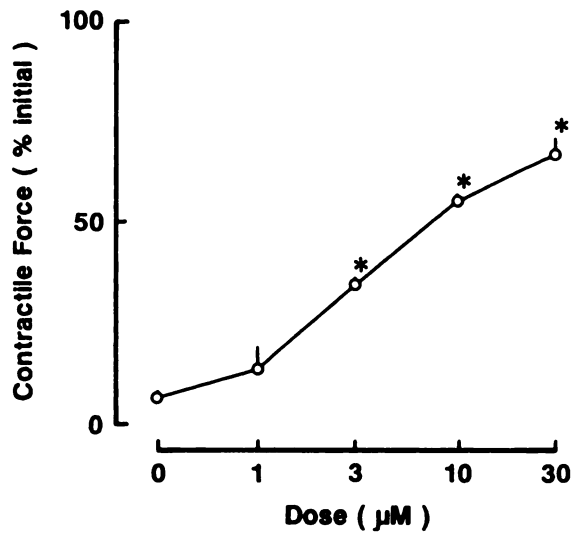

Fig. 6. Relationship between recovery of cardiac contractile force of the perfused heart after reoxygenation and doses of yohimbine administered during hypoxic period. Statistical significance was estimated by Dunnett's t test ($P < .05$). *Significantly different from control (without yohimbine treatment).

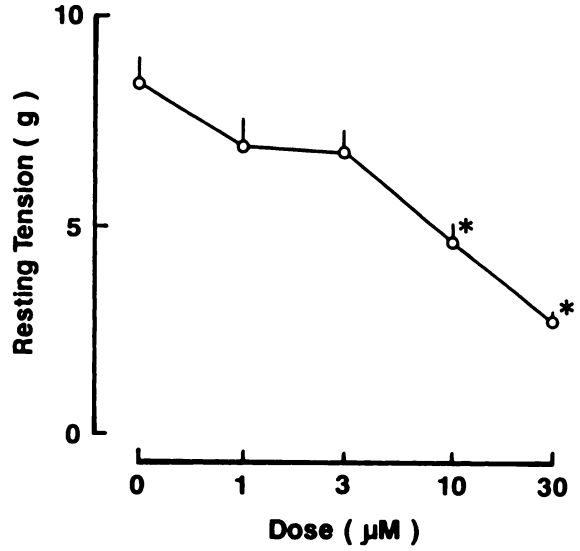

Fig. 7. Relationship between resting tension of the perfused heart at 45 min of reoxygenation and doses of yohimbine administered during hypoxic period. Statistical significance was estimated by Dunnett's t test ($P < .05$). *Significantly different from control (without yohimbine treatment).

Tokyo, Japan). After 25 min of equilibration with the normal Krebs-Henseleit solution, the hearts were perfused with hypoxic medium, previously gassed with 95% N_2 + 5% CO_2. In the hypoxic medium, glucose was replaced with 11 mM Tris/HCl to rapidly reduce the substrates for anaerobic glycolysis. Treatment with yohimbine at concentrations ranging from 1 to 30 μM was commenced after 5 min of hypoxic perfusion with an infusion rate of 0.1 ml/min through a needle introduced into the aortic cannula by means of a microtube pump (Terumo STC-521, Terumo Co., Tokyo, Japan). Infusion of yohimbine was continued throughout 20 min of hypoxic perfusion. The hearts were reoxygenated by perfusion for 45 min with normal Krebs-Henseleit solution containing glucose and equilibrating with a gas mixture of 95% O_2 + 5% CO_2 as described above. The hearts were paced throughout perfusion sequence, except for the first 10 min of reoxygenation, to prevent arrhythmias which frequently occur during this period. The PO_2 of the hypoxic and reoxygenated perfusion buffers was less than

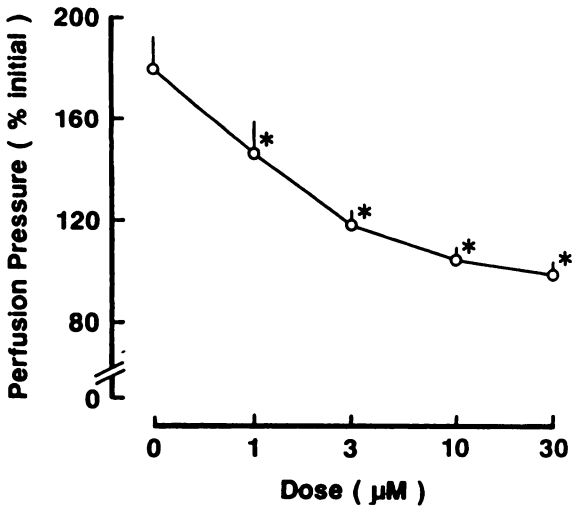

Fig. 8. Relationship between perfusion pressure of the perfused heart at 45 min of reoxygenation and doses of yohimbine administered during hypoxic period. Statistical significance was estimated by Dunnett's *t* test ($P < .05$). *Significantly different from control (without yohimbine treatment).

28 mm Hg and more than 610 mm Hg, respectively, measured at the aortic cannula. Control hearts were perfused for 20 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.

The following perfusion protocol was performed: A) preperfusion, hearts perfused for 25 min for equilibration ($n = 4$); B) normoxia for 20 min, hearts perfused for 20 min under glucose-free, normoxic conditions with ($n = 6$) and without 30 μM yohimbine-treatment ($n = 5$); C) normoxia for 65 min, hearts perfused for 45 min under normoxic conditions following 20 min glucose-free, normoxic perfusion with ($n = 6$) and without 30 μM yohimbine-treatment ($n = 6$); D) hypoxia for 20 min, hearts perfused for 20 min under hypoxic conditions with ($n = 7$) and without 30 μM yohimbine-treatment ($n = 6$); E) hypoxia and reoxygenation, hearts perfused for 45 min under reoxygenated conditions following 20 min hypoxic perfusion without ($n = 10$) and with 1 ($n = 5$), 3 ($n = 5$), 10 ($n = 5$) and 30 μM ($n = 6$) yohimbine treatment.

Determination of creatine kinase activity and ATP metabolites in the perfusate. At the end of the perfusion sequence, hearts were released from the perfusion apparatus and examined biochemically. The perfusate was collected throughout the experiment. Creatine kinase activity of the perfusate was measured by the methods of Bergmeyer *et al.* (1970).

To determine ATP metabolites in the perfusate, the perfusate collected was also analyzed by high performance liquid chromatography. Purine nucleosides and their metabolites were separated through a column of C18-cellulose acetate (Cosmosil 5C18, Nakarai Tesque, Koyto, Japan) with 4.6 mm diameter and 15 cm length, by an elution with 0.25 M KH_2PO_4 containing 4% CH_3CN (pH 6.25) at a flow rate of 1 ml/min according to the method described previously (Takeo *et al.*, 1988).

Myocardial high-energy phosphates. At the end of appropriate perfusion sequence, a small apical portion of the left ventricle was frozen quickly in liquid nitrogen. The frozen tissue was weighed and then pulverized in a stainless steel tube with a stainless steel plunger under liquid nitrogen cooling. Myocardial metabolites were extracted with 0.3 M $HClO_4$ + 0.25 mM EDTA. The extract was neutralized with 2.5 M K_2CO_3, then centrifuged at 1000 × *g* for 20 min. The resultant supernatant fluid was used as a sample for determination of ATP and CP. Measurement of ATP content was carried out by the enzymatic methods described previously (Takeo *et al.*, 1988). CP content was determined according to the methods of Lowry and Passonneau (1972). To determine the ratio of dry tissue weight to frozen tissue weight, a piece of frozen tissue was weighed and dried at 120°C for 15 hr. Then, the dry tissue weight was estimated. The mean value for dry tissue weight was 11.5 ± 0.3% of the corresponding frozen tissue weight ($n = 71$).

Tissue ion and water contents. At the end of appropriate perfusion sequence, 20 ml of 320 mM sucrose/20 mM Tris/HCl (pH 7.4) was infused into the myocardium *via* the aortic cannula to eliminate the perfusing solution from vascular space, according to the method of Alto and Dhalla (1979). About 100 mg of the left ventricle was cut into eight pieces, weighed and dried at 120°C for 15 hr. After estimating the dry tissue weight, the pieced myocardium was suspended in 1 ml of concentrated HNO_3 and heated to dryness at 180°C for 30 min. The ashy myocardium was resuspended in 2.5 ml of 0.75 N HNO_3 and centrifuged at 1000 × *g* for 20 min. The supernatant fluid was diluted twice with 10 mM $LaCl_3$/0.1 N HCl for determination of tissue calcium or diluted 40 times with double-distilled, deionized water for determination of tissue sodium content. The ion contents were analyzed by an atomic absorption spectrophotometer (Shimazu AA-646, Kyoto, Japan).

Statistics. Results were expressed as the mean ± S.E.M. Student's *t* test was employed for comparison of values between each group, and Dunnett's *t* test for comparison of values in the dose-response study. Difference at the 95% confidence level was considered significant ($P < .05$).

Results

Effects on maximal driving frequency. In the first set of experiments, we determined the effects of yohimbine on the maximal driving frequency of guinea pig left atria, as an index of its action on the effective refractory period of cardiac muscle cells (Vaughan Williams and Szekeres, 1961; Levy and Richards, 1965; Basil *et al.*, 1973). As shown in figure 1, yohimbine at concentrations of 3 μM or greater significantly reduced the maximal driving frequency of the left atria.

TABLE 1

Tissue high-energy phosphates of rabbit hearts perfused under hypoxic and reoxygenated conditions with and without yohimbine treatment

Values are expressed as μmol/g dry tissue. Each value represents the mean ± S.E.M. Numbers of experiments are shown under "Methods." The initial ATP and CP contents in the myocardium (at 0 min of perfusion) were 25.74 ± 1.53 and 39.14 ± 2.16 μmol/g dry tissue ($n = 4$), respectively.

	20-min Perfusion		65-min Perfusion	
	ATP	CP	ATP	CP
Normoxia				
Without yohimbine	20.11 ± 0.60	15.33 ± 1.00	25.42 ± 0.66	35.59 ± 0.69
With yohimbine	19.35 ± 1.21	18.29 ± 0.91	24.04 ± 0.99	34.06 ± 0.80
Hypoxia and reoxygenation				
Without yohimbine	6.23 ± 0.15†	5.85 ± 0.20†	8.12 ± 0.65†	13.93 ± 0.68†
With yohimbine	5.96 ± 0.07†	5.88 ± 0.07†	20.91 ± 0.24†*	30.02 ± 1.14†*

* Significantly different from the corresponding group without yohimbine treatment ($P < .05$).
† Significantly different from the corresponding normoxic group.

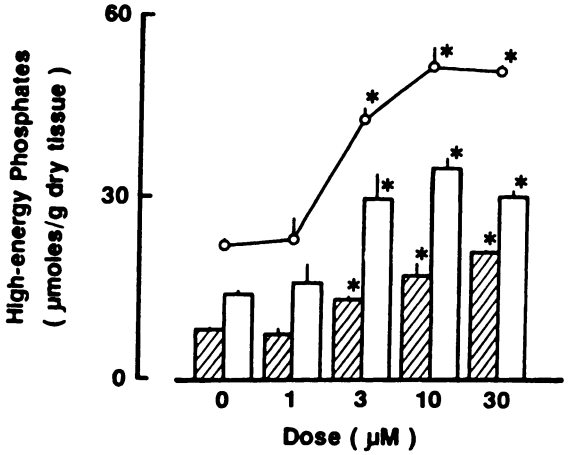

Fig. 9. Relationship between myocardial ATP (▨), CP (□) and total high-energy phosphate (O) of the perfused heart at 45 min of reoxygenation, and doses of yohimbine administered during hypoxic period. Statistical significance was estimated by Dunnett's *t* test ($P < .05$). *Significantly different from control (without yohimbine treatment).

Cardiac function of the perfused heart. In the next set of experiments, rabbit hearts were subjected to 20 min of hypoxia, followed by a 45-min reoxygenated perfusion. Functional and metabolic changes after hypoxia, and reoxygenation with and without various doses of yohimbine, were examined. Typical tracings of the perfused rabbit heart are shown in figure 2. Cardiac contractile force generated with the initial resting tension of 1.5 g (control value) was 8.4 ± 0.3 g and perfusion pressure was 22.3 ± 0.4 mm Hg ($n = 71$). Time course of changes in cardiac contractile force, resting tension and perfusion pressure of perfused rabbit hearts with and without 30 μM yohimbine treatment are shown in figures 3, 4 and 5, respectively. When the hearts were switched to the hypoxic perfusion, an immediate decline in cardiac contractile force and perfusion pressure was observed. Five min after the onset of hypoxic perfusion, the cardiac contractile force declined to 28 ± 3% of the initial developed tension ($n = 44$). The resting tension began to rise after approximately 5 min of the hypoxic perfusion and reached 9.9 ± 0.5 g (6.6-fold; $n = 16$) at 20 min of hypoxia. Cardiac contractile force was almost zero after 8 min of hypoxic perfusion. Perfusion pressure of isolated hearts declined immediately after the onset of hypoxia, followed by a gradual increase at the final stage of hypoxic perfusion. The perfusion pressure was significantly higher than the initial level after 20 min of hypoxia (182 ± 8% of the prehypoxic level; $n = 16$). Subsequent reoxygenation of the heart resulted in little recovery of cardiac contractile force (6.6 ± 0.7% of the prehypoxic level; $n = 10$) and sustained high resting tension (8.4 ± 0.6 g; $n = 10$). The perfusion pressure was raised after 45 min of reoxygenation (180 ± 13% of the prehypoxic level; $n = 10$).

Under these pathophysiological conditions, we perfused hearts with yohimbine at concentrations ranging from 1 to 30 μM for the final 15 min of the hypoxic perfusion. Treatment with cardioprotective agents only during this period minimizes the energy sparing effects of these agents (Takeo *et al.*, 1989). As shown in the lower panel of figure 2 as well as in figure 3, 30 μM yohimbine did not modify the changes in cardiac contractile force during the hypoxic period, whereas hypoxia-induced increase in perfusion pressure and rise in resting tension were significantly suppressed (119 ± 6% of the prehypoxic level and 5.09 ± 0.62 g, respectively; $n = 13$) as shown in Figures 4 and 5. This treatment also elicited a marked recovery of cardiac contractile force (68 ± 3% of the prehypoxic level; $n = 6$) and a profound suppression of the rise in resting tension (2.8 ± 0.3 g; $n = 6$) upon reoxygenation. Likewise, an increase in perfusion pressure at the final stage of reoxygenated perfusion was suppressed by treatment with 30 μM yohimbine (99 ± 6% of the prehypoxic level; $n = 6$).

The relationship between recovery of cardiac contractile force and the concentrations of yohimbine administered was examined (fig. 6). The recovery of cardiac contractile force after reoxygenation was enhanced with increasing concentrations of yohimbine administered during hypoxic perfusion. A significant recovery of the contractile force, when estimated with Dunnett's *t* test, was seen at concentrations ranging from 3 to 30 μM yohimbine. Suppression of the rise in resting tension after reoxygenation was also seen with increasing yohimbine concentrations (fig. 7). A significant suppression was observed at 10 and 30 μM yohimbine. Suppression of the increase in perfusion pressure after reoxygenation was also concentration-dependent in the range of 1 to 30 μM yohimbine (fig. 8).

In normoxic hearts, contractile force of control hearts (without yohimbine treatment) declined after 20 min of glucose-free perfusion, due to lack of substrates for energy production (fig. 3). However, force development recovered completely to the initial level after 45 min of reperfusion. The perfusion pressure and resting tension of normoxic control hearts were unaltered after 20 min of glucose-free perfusion and the following 45 min of reperfusion (figs. 4 and 5). Perfusion of normoxic hearts with 30 μM yohimbine caused a significant decrease in cardiac contractile force and a slight rise in resting tension during the 20-min glucose-free perfusion. However, these parameters returned to initial, or near initial, levels after 45 min of reperfusion.

Changes in tissue high-energy phosphates. As demon-

TABLE 2

Tissue calcium and sodium contents of rabbit hearts after hypoxic and reoxygenated perfusion with and without yohimbine treatment

Values are expressed as μmol/g dry tissue. Each value represents the mean ± S.E.M. Numbers of experiments are shown under "Methods."

	20-min Perfusion		65-min Perfusion	
	Ca	Na	Ca	Na
Normoxia				
Without yohimbine	2.67 ± 0.18	83.4 ± 10.4	2.61 ± 0.24	79.5 ± 2.9
With yohimbine	2.16 ± 0.18	104.5 ± 5.2	2.69 ± 0.28	103.9 ± 11.0
Hypoxia and reoxygenation				
Without yohimbine	3.50 ± 0.35†	120.6 ± 21.2†	5.09 ± 0.32†	158.6 ± 9.5†
With yohimbine	2.38 ± 0.12*	87.2 ± 3.3*	3.12 ± 0.45*	92.5 ± 7.1*

* Significantly different from the corresponding group without yohimbine treatment ($P < .05$).
† Significantly different from the corresponding normoxic group.

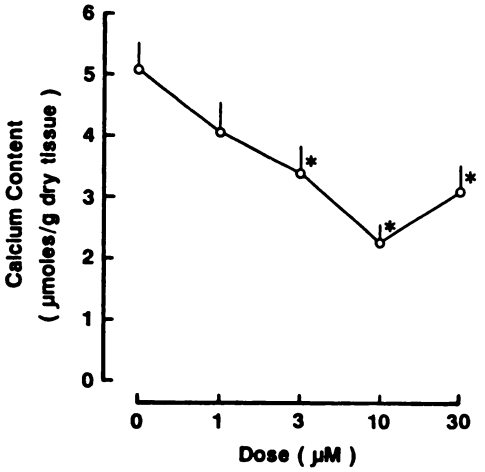

Fig. 10. Relationship between tissue calcium contents of the perfused heart at 45 min of reoxygenation and doses of yohimbine administered during hypoxic period. Statistical significance was estimated by Dunnett's *t* test ($P < .05$). *Significantly different from control (without yohimbine treatment).

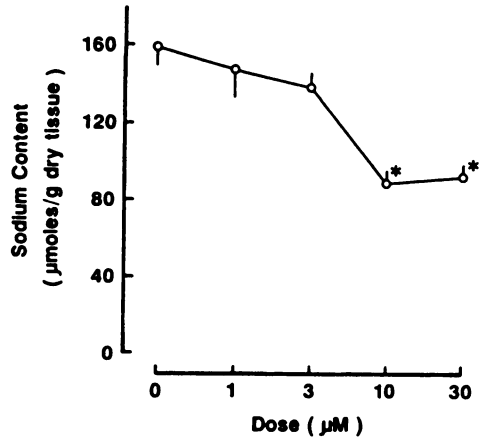

Fig. 11. Relationship between tissue sodium contents of the perfused heart at 45 min of reoxygenation and doses of yohimbine administered during hypoxic period. Statistical significance was estimated by Dunnett's *t* test ($P < .05$). *Significantly different from control (without yohimbine treatment).

strated in previous studies (Takeo *et al.*, 1988, 1989), hypoxic perfusion induced a marked reduction in myocardial high-energy phosphate contents, which were only partially restored even after 45 min of reoxygenation. As shown in table 1, myocardial high-energy phosphate levels before (0 min) and after hypoxia, and after reoxygenation, were similar to the previous results (Takeo *et al.*, 1989). There were no significant changes in the myocardial high-energy phosphate contents of the normoxic hearts after 20 min of glucose-free perfusion or following a 45-min reperfusion, regardless of yohimbine treatment. Yohimbine, at the concentrations of 3 μM or greater, resulted in a significant restoration of myocardial high-energy phosphate contents after reoxygenation (fig. 9).

Changes in tissue calcium and sodium contents. We have also determined tissue calcium and sodium contents of the perfused rabbit hearts. Table 2 indicates tissue ion contents of hypoxic and reoxygenated myocardium with and without 30 μM yohimbine treatment. Myocardial calcium content increased slightly, but significantly, during the 20-min hypoxic period. A marked increase in tissue calcium was seen when the heart was reoxygenated for 45 min (about 2-fold increase rela-

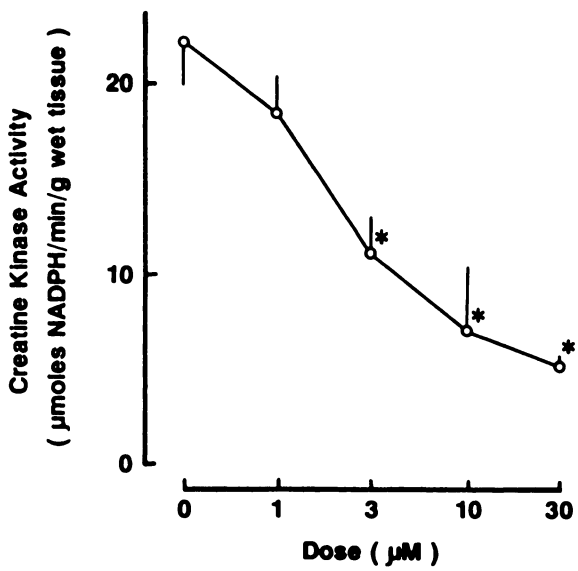

Fig. 12. Relationship between release of creatine kinase from the heart during the whole period of perfusion and doses of yohimbine administered during hypoxic period. Statistical significance was estimated by Dunnett's *t* test. *Significantly different from control (without yohimbine treatment).

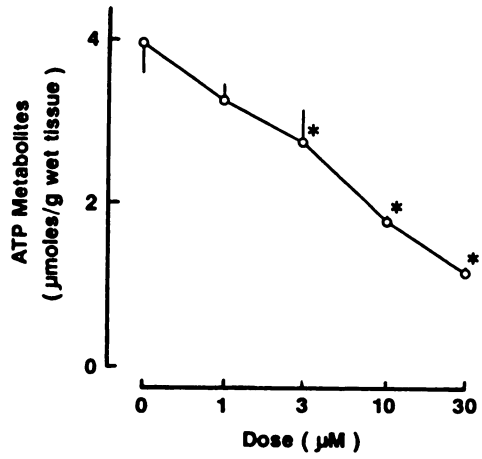

Fig. 13. Relationship between release of ATP metabolites from the hearts during the whole period of perfusion and doses of yohimbine administered during hypoxic period. Statistical significance was estimated by Dunnett's *t* test ($P < .05$). *Significantly different from control (without yohimbine treatment).

TABLE 3

The effects of *alpha* blockers and antiarrhythmic agents on the maximal driving frequency of the guinea pig left atria

Values are expressed as beats/min. Concentrations in parentheses indicate doses used in the isolated rabbit heart preparations.

Agents	*n*	Without Agent (control)	With Agent
Phentolamine (83 μM)	7	664 ± 26	479 ± 23*
Bunazosin (46 μM)	6	662 ± 29	248 ± 24*
YM-12617 (28 μM)	6	611 ± 26	514 ± 12*
Amosulalol (45 μM)	7	696 ± 12	462 ± 27*
Lidocaine (69 μM)	7	681 ± 43	417 ± 27*
Disopyramide (55 μM)	6	680 ± 12	495 ± 12*

* Significantly different from control ($P < .05$). Control values (without agents) were not different from each other when statistically evaluated by analysis of variance followed by Scheffe *t* test.

tive to the prehypoxic level). A significant increase in tissue sodium content was seen after 20 min of hypoxic perfusion. The tissue sodium content increased 2-fold after reoxygenation relative to the initial value. Treatment of hypoxic hearts with 30 μM yohimbine significantly attenuated these changes in tissue calcium and sodium. In normoxic hearts, there were no significant changes in the tissue calcium and sodium at the 20-min and subsequent 45-min perfusions.

We focused on the relationship between reoxygenation-induced recovery of cardiac contractile force and altered tissue calcium and sodium contents at 45 min of reoxygenation in rabbit hearts treated with different concentrations of yohimbine. The results are shown in figures 10 and 11, respectively. Attenuation of reoxygenation-induced increase in tissue calcium was elicited when the heart was treated with yohimbine (3 μM or greater). Concentration-dependent suppression in tissue sodium content was seen in the perfused hearts after reoxygenation. Yohimbine, at concentrations of 10 and 30 μM, restored tissue sodium to near prehypoxic levels.

Tissue water content of the perfused hearts was not substantially altered by any perfusion protocol or any intervention used. For example, the tissue water contents of the prehypoxic, hypoxic and reoxygenated hearts were 75.9 ± 0.5 (n = 4), 78.0 ± 0.6 (n = 6) and 76.9 ± 0.6% (n = 10), respectively, and those of yohimbine-treated hearts at 20 min and 65 min of perfusion were 77.2 ± 1.0 (n = 7) and 76.5 ± 1.2% (n = 6), respectively.

Changes in creatine kinase activity of the perfusate. The perfusate eluted from hypoxic and reoxygenated hearts was collected and the creatine kinase released into the perfusate was determined. The release of creatine kinase from normoxic hearts during the 65-min perfusion period was 1340 ± 232 nmol NADPH/min/g wet tissue (n = 6). There was a slight release of creatine kinase from normoxic hearts when treated with 30 μM yohimbine (1617 ± 246 nmol/min/g wet tissue; n = 5). A marked release of creatine kinase from perfused hearts was seen by hypoxia/reoxygenation (22216 ± 2285 nmol NADPH/min/g wet tissue; n = 10). Treatment of hypoxic hearts with yohimbine at concentrations of 3 μM or greater resulted in a profound suppression of the release of creatine kinase from hypoxic and reoxygenated hearts (fig. 12).

Changes in ATP metabolites of the perfusate. The perfusate from hypoxic and reoxygenated hearts with or without yohimbine treatment was collected and the amount of ATP metabolites in the perfusate was determined by high performance liquid chromatography. Hypoxia and subsequent reoxygenation elicited a marked increase in the release of hypoxanthine, inosine and adenosine, and consequently a significant increase in the release of total ATP metabolites (4126 ± 399 nmol/g wet tissue; n = 10). The effect of varying concentrations of yohimbine on the release of ATP metabolites was examined and the results are shown in figure 13. Yohimbine (3–30 μM) significantly suppressed the release of ATP metabolites. In normoxic hearts with and without yohimbine treatment, there were no significant differences in the release of ATP metabolites during the 65-min perfusion period (184 ± 64 and 128 ± 45 nmol/g wet tissue, respectively, n = each 6).

Effects of *alpha* blockers and antiarrhythmic agents on maximal driving frequency. The relationship between cardioprotective agents used in previous studies on posthypoxic contractile recovery of isolated perfused hearts and effects of *alpha* adrenoceptor blockers and antiarrhythmic agents on the maximal driving frequency of guinea pig left atria were examined. The results are shown in table 3. Significant decreases in the maximal driving frequency of the left atria were seen when phentolamine, bunazosin, amosulalol, YM-12617, lidocaine and disopyramide were applied to the guinea pig left atrial preparations at the same concentrations as those used in the isolated perfused rabbit hearts subjected to hypoxia/reoxygenation. It should be mentioned that the posthypoxic recovery of cardiac contractile force after treatment with phentolamine, bunazosin, amosulalol, YM-12617, lidocaine and disopyramide, when determined in isolated rabbit heart preparations perfused in the same manner or in a manner similar to the present study, were 59, 57, 83, 90, 75 and 75% of each prehypoxic level, respectively (Takeo *et al.*, 1989; Tanonaka *et al.*, 1989a,b), indicating significant improvement of posthypoxic recovery of cardiac contractile force by treatment with these agents.

Discussion

In the present study we have shown that yohimbine, at concentrations of 3 μM or greater, elicited a significant recovery of cardiac contractile force and resting tension of perfused hearts upon reoxygenation, after a period of hypoxic perfusion. The recovery was associated with restoration of myocardial high-energy phosphates which had been profoundly depleted during hypoxic perfusion, and inhibition of release of ATP metabolites and creatine kinase from the perfused heart. It was also associated with a suppression of the rise in tissue sodium and calcium during hypoxia and reoxygenation. It should be emphasized that posthypoxic recovery of most of these functional and metabolic variables was concentration-dependent (figs. 6–13). Yohimbine, therefore, is capable of exerting beneficial effects on the oxygen-deprived and subsequently oxygen-replenished heart.

A loss of purine nucleosides from hearts during ischemia/reperfusion or hypoxia/reoxygenation has been shown to be one of the critical factors in the induction of myocardial cell damage (Kloner *et al.*, 1981; Reimer *et al.*, 1989; Vary *et al.*, 1979). Furthermore, ATP metabolites such as adenosine and inosine are substrates for the salvage synthesis of ATP when oxygen is replenished in the myocardial cell. Thus, preservation of ATP metabolites in the myocardial cell during hypoxic and reoxygenated perfusion is of great importance in the restoration of myocardial high-energy phosphates upon reoxygenation.

A release of creatine kinase from oxygen-deprived hearts is well recognized to occur due to myocardial cell necrosis and/or changes in cell membrane permeability (Ganote and Kaltenbach, 1979). Thus, a suppression of the release of creatine kinase by yohimbine treatment is also an indicator of myocardial protection.

An increase in tissue sodium was observed during hypoxic perfusion, whereas an increase in tissue calcium during hypoxic perfusion was small, though significant. Tissue calcium, however, increased markedly upon reoxygenation. This is consistent with the view of Tani and Neely (1989) who proposed that an increase in tissue sodium during ischemia may enhance tissue calcium gain through sodium-calcium exchange upon reperfusion. Tissue calcium overload has been proposed to mediate damage to the cardiac cell through activation of phospholipases (Chien *et al.*, 1979) and neutral proteases (Reddy *et al.*, 1974). Thus, ischemia/reperfusion or hypoxia/reoxygenation may result in serious damage to cardiac function and myocardial metabolism due to calcium overload. Accordingly,

suppression of the tissue ion disturbance by treatment with yohimbine may be one of the mechanisms by which it protects the hypoxic and posthypoxic heart.

A recent study from our laboratory (Takeo *et al.*, 1990) has shown that cardioprotective effects of propranolol and acebutolol, but not atenolol or metoprolol, are closely related to their effects on the maximal driving frequency of left atrial muscles. A similar relationship was also observed in the present study. That is, yohimbine, at concentrations ranging from 3 to 30 μM, significantly inhibited the maximal driving frequency. Yohimbine, in the same concentration range, enhanced recovery of cardiac contractile force and myocardial metabolism upon reoxygenation. As described earlier, we have observed a significant enhancement of posthypoxic contractile and metabolic recovery of the reoxygenated myocardium after treatment with several *alpha* blocking agents such as phentolamine, bunazosin, amosulalol and YM-12617, and *beta* blocking agents such as propranolol and acebutolol. In the present study, we found that the above agents, at the same concentrations, inhibited the maximal driving frequency of left atrial muscle as shown in table 3. Thus, there is an apparent relationship between posthypoxic functional and metabolic recovery of perfused hearts and depression in the maximal driving frequency of atrial muscles.

Influence of agents on the maximal driving frequency of atria is indicative of their effect on the effective refractory period of cardiac muscles (Dawes, 1946; Levy and Richards, 1965; Vaughan Williams and Szekeres, 1961), although other factors may also contribute to this effect (Dawes and Vane, 1956), It would seem, therefore, that yohimbine and the other *alpha* blocking agents studied here may prolong the effective refractory period of cardiac muscle. Prolongation of the effective refractory period is thought to be, at least in part, caused by an inhibition of sodium flux (Vaughan Williams, 1958; Wojtczak and Beresewics, 1974; Chen *et al.*, 1975; Carmeliet *et al.*, 1976). Thus, it is likely that depression of the maximal driving frequency is associated with an inhibition of transmembrane flux of sodium through its channel. In contrast, 3 mM yohimbine has been shown to exhibit local anesthetic action (Shaw *et al.*, 1955; Simon, 1955) and block fast sodium channel of the embryonic chick heart at concentrations of 10 to 100 μM (Azuma *et al.*, 1978). These concentrations are similar to those which depressed maximal driving frequency of the guinea pig left atria. This strongly supports the suggestion that the observed beneficial effects on post hypoxic contractile and metabolic function are not attributable to *alpha*-2 adrenoceptor blocking action, but rather related to an action of transmembrane flux of sodium. In a previous study (Takeo *et al.*, 1989), we have shown that the class I-type antiarrhythmic agents, lidocaine and disopyramide, enhanced posthypoxic recovery of cardiac contractile force and metabolism in perfused rabbit hearts subjected to hypoxia and reoxygenation, under experimental conditions similar to the present study. Furthermore, we observed that both lidocaine and disopyramide are also effective in depressing the maximal driving frequency of left atrial muscle as shown in table 3. This adds further support to our suggestion that these effects are related.

Yohimbine has been shown to enhance catecholamine release in various tissues such as brain (Frankhuyzen and Mulder, 1982), vas deferens (Belis *et al.*, 1982) and pulmonary artery (Starke *et al.*, 1975) with concentrations ranging from 0.01 to 1 μM. This suggests that 1 μM yohimbine is sufficient to exert *alpha*-2 adrenoceptor blocking action. In the present study, however, 1 μM yohimbine did not produce an appreciable posthypoxic recovery of cardiac contractile force or metabolic function. This suggests that the *alpha*-2 adrenoceptor blocking action is not involved in the posthypoxic effect. In addition, to our knowledge, there is little evidence for the presence of *alpha*-2 adrenoceptor-mediated action in the myocardium. The role of *alpha*-2 adrenoceptors in coronary vessel function has not been established at present. It would seem, therefore, that *alpha*-2 adrenoceptor-mediated action of yohimbine does not play a role in the improvement of the posthypoxic recovery of cardiac function and metabolism observed in the present study.

Recently, Mak and Weglicki (1988) have shown that *beta* blocking agents such as propranolol, pindolol, atenolol and sotalol, at high concentrations (20 and 200 μM), are capable of protecting the cardiac sarcolemmal membrane against free radical peroxidation *in vitro*. Because the lower concentration used in their study is similar to that of yohimbine, *beta* blocking agents and *alpha* blocking agents in our experiments, the beneficial effects may be related to such antifree radical-mediated damage to cardiac sarcolemmal membrane. Metoprolol and atenolol, however, did not protect the myocardium against hypoxia/reoxygenation damage, although they protected against membrane peroxidation. Conversely, lidocaine did protect against hypoxia/reoxygenation damage but did not protect against free radicals. It seems unlikely, therefore, that the cardioprotective effects of agents used in this study are due solely to prevention of lipid peroxidation.

In conclusion, we have shown in the present study that yohimbine, at concentrations which depressed the maximal driving frequency of heart muscle, exerts beneficial effects on the posthypoxic contractile and metabolic function in isolated perfused rabbit hearts. Such effects were also observed in hearts treated with several *alpha* blocking and *beta* blocking agents at concentrations which depress the maximal driving frequency. Depression of maximal driving frequency, prolongation of refractory period or inhibition of fast sodium channel is one of the indicators of specific membrane activity, a redefined one of so-called "membrane stabilizing activity" (Smith, 1982). Thus, an agent which has such activity may prevent hypoxia/reoxygenation-induced damage to the myocardium. This mechanism, however, does not explain all the clinical and experimental effects of *alpha* and *beta* blocking agents on the ischemic and hypoxic myocardium. Furthermore, the concentrations of agents necessary to achieve the cardioprotective effects as described above are relatively high. Thus, we would rather emphasize that this effect on the cardiac cell membranes is one of the possible mechanisms by which these agents protect the myocardium from functional and metabolic derangement following oxygen deficiency and subsequent oxygen replenishment.

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