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Cardioprotective and Antioxidant Effects of Apomorphine

IGOR KHALIULIN[a], JOSEPH B. BORMAN[a], MORDECHAI CHEVION[b,*] and HERZL SCHWALB[a]

[a]*The Joseph Lunenfeld Cardiac Surgery Research Center, Hadassah University Hospital, Jerusalem, Israel;* [b]*Department of Cellular Biochemistry and Human Genetics, The Hebrew University-Hadassah Schools of Medicine and Dental Medicine, P.O. Box 12272, 91120 Jerusalem, Israel*

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Apomorphine is a potent antioxidant that infiltrates through biological membranes. We studied the effect of apomorphine (2 μM) on myocardial ischemic-reperfusion injury in the isolated rat heart. Since iron and copper ions (mediators in formation of oxygen-derived free radicals) are released during myocardial reperfusion, apomorphine interaction with iron and copper and its ability to prevent copper-induced ascorbate oxidation were studied. Apomorphine perfused before ischemia or at the commencement of reperfusion demonstrated enhanced restoration of hemodynamic function (i.e. recovery of the work index (LVDP × HR) was 69.2 ± 4.0% with apomorphine pre-ischemic regimen vs. 43.4 ± 9.01% in control hearts, $p < 0.01$, and 76.3 ± 8.0% with apomorphine reperfusion regimen vs. 30.4 ± 11.1% in controls, $p < 0.001$). This was accompanied by decreased release of proteins in the effluent and improved coronary flow recovery in hearts treated with apomorphine after the ischemia. Apomorphine forms stable complexes with copper and with iron, and inhibits the copper-induced ascorbate oxidation. It is suggested that these iron and copper chelating properties and the redox-inactive chelates formed by transition metals and apomorphine play an essential role in post-ischemic cardioprotection.

Keywords: Myocardial ischemia; Reperfusion; Hemodynamics; Radicals; Copper and iron chelation

INTRODUCTION

Hearts subjected to prolonged ischemia lose a considerable fraction of their function due to ischemic and reperfusion injury. A critical role in this injury is attributed to the formation of oxygen-derived reactive species.[1] The formation of reactive oxygen species from relatively less reactive species seems to be mediated by redox-active metal ions.[1,2] This burst of transition metals leakage correlates well with the degree of loss of cardiac function and with the redox activity of the metals during early reperfusion.[3] Thus, the release of redox-active transition metals at the commencement of reperfusion plays an important role in the myocardial injury. The biochemical mechanisms responsible for ischemic and reperfusion injury are many and varied[4] but they include the generation of hydrogen peroxide (H_2O_2) and the superoxide radical ($^{\cdot}O_2^-$).[5,6] $^{\cdot}O_2^-$ and H_2O_2 form a highly reactive species that can attack and destroy almost all known biomolecules.[4] This species is the hydroxyl radical ($^{\cdot}OH$), and its formation requires traces of transition metal ions. $^{\cdot}OH$ is formed via the site-specific Haber–Weiss reaction.[1,3] Iron chelation has been shown to protect against tissue injury following ischemia,[7–10] while addition of iron and copper to the perfusate facilitated injury in hearts subjected to ischemia and reperfusion.[11,12]

Apomorphine, widely used in the treatment of Parkinson's disease,[13] is a potent antioxidant.[14] It possesses iron-chelating properties[15] and is capable of penetrating through biological membranes into the cell.[16] Extensive efforts have been devoted to study the effect of apomorphine on brain function, mediators release and behavior. There are also publications on the effects of apomorphine on cardiac

*Corresponding author. Tel.: +972-2-6758160. Fax: +972-2-6415848. E-mail: chevion@cc.huji.ac.il

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and circulatory functions.[17,18] However, to our knowledge, this drug has not been studied in the heart exposed to ischemia and reperfusion.

Thus, the aim of the present study was to test the capability of apomorphine to attenuate ischemic and reperfusion damage to the heart and suggest a possible mechanism of its action.

MATERIALS AND METHODS

Chemicals

Apomorphine, ferric ammonium sulfate {$Fe(NH_4)(SO_4)_2 \cdot 12H_2O$}, and nitrilotriacetic acid (NTA) were obtained from Sigma Chemical Co. (St. Louis, MO). *O*-phenanthroline was obtained from Fluka Chemie AG (Buchs, Switzerland).

Isolated Heart Perfusion

Male Sprague–Dawley rats weighing 280 ± 20 g were used for perfusion experiments. Handling of the animals was in accord with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The animals were injected, intraperitoneally, with sodium heparin (500 U) and 30 min later were anesthetized with pentobarbital (30 mg/animal). The hearts were immediately removed and placed in heparinized ice-cold saline solution. The aorta was cannulated to a Langendorff perfusion apparatus and the pulmonary artery was cut open to provide drainage.

Retrograde aortic perfusion was maintained with modified Krebs–Henseleit (KH) solution according to Neely and Rovetto[19] containing: 118 mM NaCl, 4.9 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 11.1 mM glucose and 2.5 mM $CaCl_2$. The KH was aerated with a mixture of 95% (v/v) oxygen and 5% (v/v) carbon dioxide. Aortic perfusion was maintained at 37°C and a pressure of 90 cm H_2O.

Apomorphine was dissolved in saline and perfused through a side arm directly into the aortic cannula by a Syringe Pump (Razel Scientific Instruments, Inc. Stamford CT). The infusion rate was adjusted to account for heart coronary flow rate and was equal to 1/20 of coronary flow for each heart. Thus, apomorphine (40 μM) was diluted in the perfusate to the final concentration 2 μM, in most experiments, unless specified otherwise. This concentration was chosen according to our preliminary study.

Experimental Protocols

The Effect of Apomorphine on Reperfusion Injury

Hearts were subjected to 35 min of perfusion, 25 min of no-flow normothermic global ischemia, and 45 min of reperfusion (control and apomorphine treated hearts). Apomorphine (2 μM) was introduced into the perfusate for 20 min during preischemia or reperfusion: Series 1 — Apomorphine was perfused for 20 min immediately prior to ischemia. Series 2—Apomorphine was perfused for 20 min starting from the onset of reperfusion.

The Effect of Apomorphine on Perfused Rat Heart (without Ischemia)

Series 3—The protocol consisted of 20 min of KH perfusion (equilibration), 20 min of KH perfusion with apomorphine (2 μM), and 30 min of KH perfusion without apomorphine.

Hemodynamic Measurements

Hemodynamic parameters were monitored using a latex balloon-tipped catheter inserted through an incision in the left atrium and advanced through the mitral valve into the left ventricle and connected to a pressure transducer placed at equivalent height to the heart, and a recording system (Hewlett Packard 7758B, USA). The balloon was inflated and equilibrated to give an end-diastolic pressure of 0 mmHg. Left ventricular systolic and diastolic pressures and time derivatives of pressure were measured during contraction ($+dP/dt$) and relaxation ($-dP/dt$). Left ventricular developed pressure (LVDP) was calculated as the difference between the systolic and diastolic pressures. The work index of heart (LVDP × HR) was derived from the product of LVDP and heart rate (HR). Coronary flow rate (CF) was measured by collecting the effluent drained through the pulmonary artery during pre-ischemia and reperfusion.

Ischemic/reperfusion Damage

Protein released from the heart during the reperfusion was considered a marker for ischemic and reperfusion damage.[20] Protein content in the perfusate was determined by the Coomassie Blue method using the Bio-Rad protein assay kit and a protein standard of bovine serum albumin. Protein concentration in coronary flow fraction of perfusate (μg/ml), coronary flow rate (ml/min) and protein release, i.e. product of protein concentration and coronary flow rate (μg/min) was measured for each 20 s of the first 2 min of reperfusion and on the fifth minute of reperfusion.

In Vitro Studies

Apomorphine—Cu (II) and Fe (III) Interaction

The interaction between apomorphine and copper or iron and the stochiometry of the corresponding

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complexes were spectrophotometrically determined according to the procedure of Nagano *et al*.[21] Briefly, increasing concentrations of apomorphine (0–0.2 mM) were mixed with the corresponding decreasing concentrations of $CuSO_4$ (0.2–0 mM) or $(Fe(III)(NTA)_2)^{3-}$ complex (0.1–0 mM) in Tris buffer (10 mM, pH 7.2) (see Fig. 7A,B). Spectra (250–450 nm) were recorded using a Uvikon XL spectrophotometer (Bio-Tek Instruments, Italy). $(Fe(III)(NTA)_2^{3-}$ complex was prepared by mixing equal volumes of $Fe(NH_4)(SO_4)_2{\cdot}12H_2O$ (0.2 mM) and NTA (0.4 mM) in Tris buffer (10 mM, pH 7.2). Apomorphine solutions were kept under nitrogen before use, in order to avoid oxidation.

The Effect of Apomorphine on Ascorbate Oxidation

Autooxidation of ascorbate can be used as a test for the presence of redox-active metals in buffers.[22] Apomorphine (50 μM) was preincubated with sodium ascorbate (5 mM), $CuSO_4$ (50 μM) and HEPES buffer (5 mM, pH 7) in a shaking water bath at 37°C (aerobic conditions). The residual ascorbate (reduced form) was measured spectrophotometrically at 515 nm using the ferri-phenanthroline reduction assay.[23] At pre-determined time intervals, 30 μl samples were withdrawn and added to optical cells containing 0.3 ml of ferriphenanthroline stock solution (1.8 mM) and 2.7 ml of 0.1 M imidazole buffer (pH 8.0), prepared as previously described.[23] The apparent first order rate constant for ascorbate oxidation (k) was calculated.

Statistical Analysis

Results are expressed as Mean ± SEM in the figures and tables. Statistical significance of differences between groups of hearts in the first and second series of experiments and in the *in vitro* experiments was evaluated using ANOVA and Mann–Whitney rank test. In the third series a Wilcoxon signed rank test was used to compare parameter change vs. the initial value. Statistical differences of $p < 0.05$ were considered to be significant.

RESULTS

Dose-response Curve of Apomorphine

As a preliminary study apomorphine (1, 2, 5 and 25 μM) was introduced in the KH at the commencement of reperfusion after the 25 min of no-flow global ischemia. Apomorphine in a concentration of 25 μM did not improve hemodynamic recovery. Lower concentration of apomorphine (1–5 μM) increased recovery of the hemodynamic parameters, and the highest recovery was achieved at the concentration of 2 μM (data not shown). Thus, the concentration of 2 μM apomorphine was chosen for the present study.

The Effect of Apomorphine on Reperfusion Injury

Series 1—Perfusion with Apomorphine During the Preischemic Phase

HEMODYNAMIC RECOVERY

Global normothermic ischemia (25 min) followed by reperfusion (45 min) led to pronounced decline of the hemodynamic function in control hearts. Adding of apomorphine to the perfusate (20 min) immediately prior to the ischemia significantly improved the post-ischemic recovery of the hemodynamic parameter i.e. the work index (LVDP × HR) in hearts treated with apomorphine recovered to 69.2 ± 4.0 vs. 43.4 ± 9.0% in control hearts ($p <$ 0.01). The heart-rate was not affected by the drug (Table I).

Post-ischemic recovery of coronary flow in hearts treated with apomorphine was similar to that of controls during the first 5 min of reperfusion (Fig. 1). However, at the end of the reperfusion the recovery was significantly higher in the apomorphine treated hearts (68.3 ± 5.0) vs. control hearts (46.0 ± 8.8) ($p < 0.05$; Table I).

TABLE I The effect of apomorphine, perfused during 20 min prior to 25 min global ischemia, on hemodynamic recovery (at 45 min of reperfusion) of isolated rat heart—first series

Group	1—Control $n = 8$		2—Pre-ischemic apomorphine for 20 min, $n = 9$	
Stage of experiment	Preischemia value	End reperfusion (%)	Preischemia value	End reperfusion (%)
LVDP (mmHg)	127 ± 15	52.5 ± 10.8	111 ± 8	80.9 ± 5.6**
$+dP/dt$ (mmHg/s)	3670 ± 581	53.0 ± 13.7	2830 ± 541	83.9 ± 6.4*
$-dP/dt$ (mmHg/s)	2380 ± 355	43.6 ± 11.1	2160 ± 197	81.2 ± 5.3**
Heart rate (HR) (beat/min)	290.0 ± 8	82.2 ± 3.5	286 ± 11	86.1 ± 4.1
LVDP × HR (mmHg beat/min)	36,600 ± 3800	43.4 ± 9.01	31,700 ± 2630	69.2 ± 4.0**
Coronary flow (ml/min)	11.9 ± 0.8	46.0 ± 8.8	12.7 ± 1.5	68.3 ± 5.0*

M ± SE—Statistically significant differences, compared to Control: * $P < 0.05$; ** $P < 0.01$. LVDP— left ventricular developed pressure; $+dP/dt$—max pressure derivative during contraction; $-dP/dt$—max pressure derivative during relaxation.

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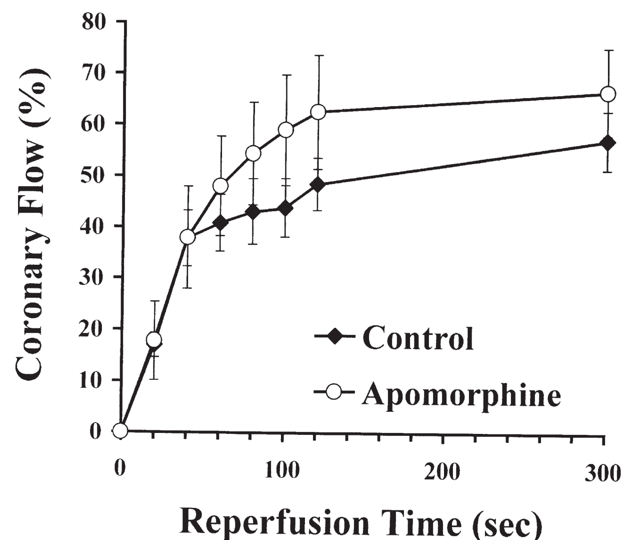

FIGURE 1 Coronary flow recovery during the first 5 min of reperfusion of control hearts ($n = 8$) and hearts treated with 2 μM apomorphine during pre-ischemic phase ($n = 9$). Coronary flow was calculated in % of pre-ischemic values, prior to the administration of apomorphine. Apomorphine was perfused for 20 min prior to 25 min of ischemia—first series.

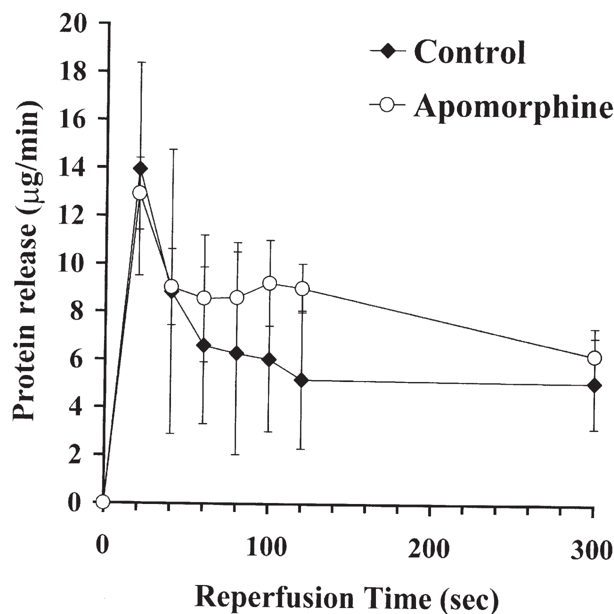

FIGURE 2 Protein released during the first 5 min of reperfusion from control hearts ($n = 8$) and hearts treated with 2 μM apomorphine in perischemic phase ($n = 9$). Apomorphine was perfused throughout 20 min prior to 25 min of ischemia—first series. No significant differences between the groups were found.

Ischemic-reperfusion Injury

Protein released from the heart during the reperfusion was used as an index for tissue damage. This index was highest during the first 20 s of reperfusion (Fig. 2). Thereafter, it gradually decreased reaching the pre-ischemic level in the fifth minute of reperfusion. The post-ischemic release of protein in apomorphine perfused hearts did not differ significantly from release in the control hearts (Fig. 2).

Series 2—Reperfusion with Apomorphine

Hemodynamic Recovery

Inclusion of apomorphine in the perfusate during the first 20 min of reperfusion considerably increased the recovery of all hemodynamic parameters, compared to control group (Table II). This recovery improvement in hearts treated with apomorphine was more pronounced than in the first series of experiments: LVDP × HR was 2.5 times higher compared to control hearts (Table II), whereas in hearts treated during pre-ischemia it was 1.6 times vs. control (Table I).

Restoration of the coronary flow, in hearts treated with apomorphine during reperfusion, was significantly faster (as early as 40 s from the start of reperfusion) than in control hearts. At 40 s of reperfusion, CF in hearts treated with apomorphine was equal to 40.2 ± 5.3% of its pre-ischemic level, whereas in control hearts it was 21.7 ± 5.5% ($p < 0.01$; Fig. 3). The improved recovery of the coronary flow in hearts treated with apomorphine persisted to the end of reperfusion (Table II, Fig. 3).

Ischemic Reperfusion Injury

Reperfusion with apomorphine caused a 50% decrease in protein released from the heart into

TABLE II Effect of apomorphine, perfused during the first 20 min of reperfusion, on hemodynamic recovery of isolated rat heart after 25 min of global ischemia and 45 min of reperfusion—second series

Group	1—Control $n = 11$		2—Apomorphine 2 μM $n = 12$	
Stage of experiment	Perischemic value	End reperfusion (%)	Preischemic value	End reperfusion (%)
LVDP mmHg	133 ± 11	41.4 ± 14.4	128 ± 8	84.5 ± 8.9**
+dP/dt mmHg/s	3430 ± 531	33.4 ± 15.8	2910 ± 493	84.1 ± 10.3**
−dP/dt mmHg/s	2270 ± 334	34.0 ± 15.2	2050 ± 466	76.3 ± 5.8**
Heart rate (HR) beat/min	282 ± 14	74.0 ± 4.5	305 ± 12	91.2 ± 7.0*
LVDP × HR mmHg × beat/min	37,300 ± 2780	30.4 ± 11.1	35,000 ± 2990	76.3 ± 8.0***
Coronary flow ml/min	10.0 ± 0.9	39.0 ± 5.3	10.3 ± 1.0	66.2 ± 3.7***

M ± SE. Statistically significant differences, compared to Control: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

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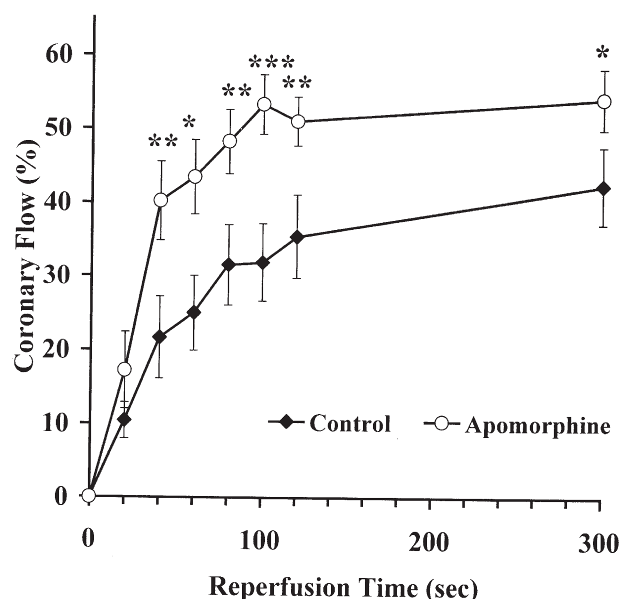

FIGURE 3 Coronary flow recovery during the first 5 min of reperfusion from control hearts ($n = 11$) and hearts treated with 2 μM apomorphine in reperfusion phase ($n = 12$). Coronary flow was calculated in % of perischemic values. Apomorphine was perfused during 20 min of reperfusion—second series.* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control.

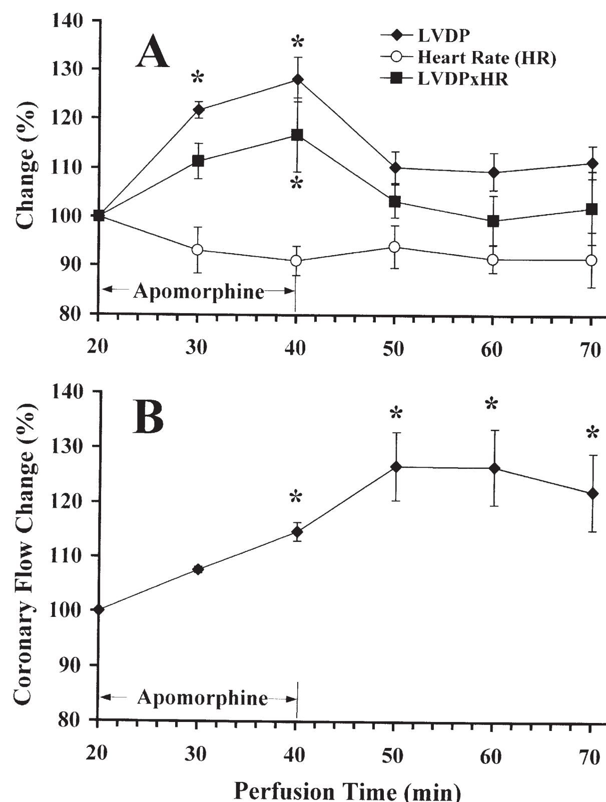

FIGURE 5 Apomorphine effect on hemodynamic parameters. Following stabilization flow on (20 min), isolated rat hearts ($n = 6$) were perfused for 20 min with apomorphine (2 μM min with KH without apomorphine—third series. A: % changes of LVDP, heart rate and LVDP × HR; B: % changes of coronary flow. * $P < 0.05$ vs. the initial value (100%) of each prior to the administration of apomorphine.

the coronary flow vs. control group, during the first 20 s of reperfusion: 10.9 + 1.5 (mg/min) in hearts treated by apomorphine vs. 19.7 + 5.2 (mg/min) in control group ($p < 0.05$, Fig. 4).

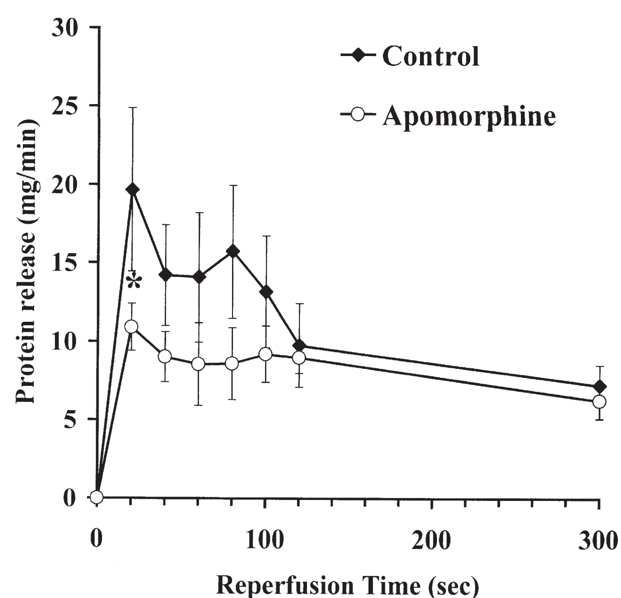

FIGURE 4 Protein released during the first 5 min of reperfusion from control heart and hearts treated by 2 μM apomorphine in reperfusion phase ($n = 12$). Apomorphine during 20 min from onset of reperfusion—second series. * $P < 0.05$ vs. control.

Effect of Apomorphine on Perfused Rat Heart (without Ischemia)

In series 3, apomorphine (2 μM) perfusion during 20 min caused a significant increase ($p < 0.05$) in the hemodynamic parameters: LVDP, LVDP × HR and CF (Fig. 5A). Cessation of apomorphine perfusion led to near initial values of the indices: LVDP (111%), and LVDP × HR (102%, Fig. 5A). In contrast, the coronary flow remained significantly increased (above 120% vs. initial value, $p < 0.05$) throughout the 30 min of drug free perfusion (Fig. 5B).

In Vitro Study

Cu(II)-Apomorphine and Fe (III)-Apomorphine Complexes

Figures 6A,B demonstrate the absorption spectra in the 250–450 nm range of Tris buffer (pH 7.2) containing Cu (II), apomorphine, and mixture of Cu (II) and apomorphine (Fig. 6A); and $(Fe(III)(NTA)_2)^{3-}$, apomorphine, and mixture of $(Fe(III)(NTA)_2)^{3-}$ and apomorphine (Fig. 6B). New specific absorbance peaks emerge in these figures: peak at 339 nm for the solution containing

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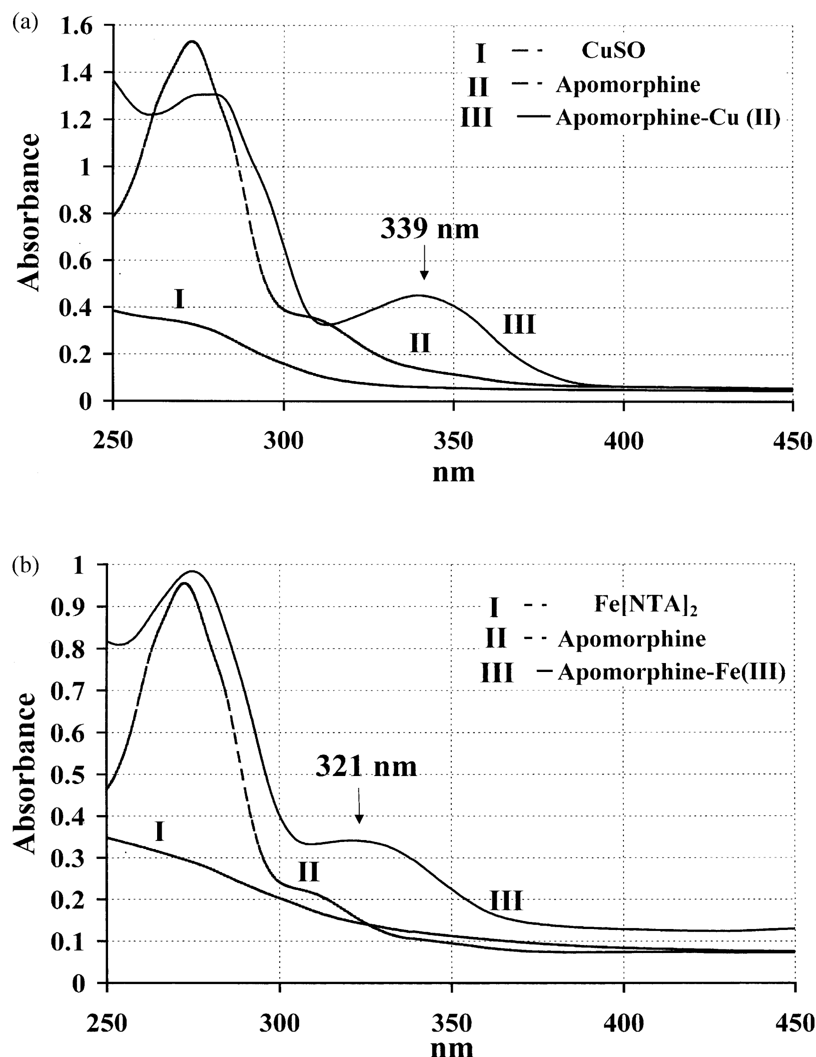

FIGURE 6 Absorbance spectra of (A): $CuSO_4$ (0.2 mM), apomorphine (0.2 mM), and mixture of $CuSO_4$ (0.1 mM) and apomorphine (0.1 mM): (B): $[Fe\ (III)(NTA)_2]^{3-}$ complex (0.2 apomorphine (0.2 mM), and mixture of $[Fe\ (III)\ (NTA)_2]^{3-}$ complex (0.1 mM) and apomorphine (0.1 mM). Solutions were prepared in Tris buffer (10 M, pH 7.2). Newly formed absorbances are marked by arrows.

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$CuSO_4$ and apomorphine, and peak at 321 nm for the solution containing $(Fe(III)(NTA)_2)^{3-}$ and apomorphine. The presence of these new peaks is attributed to the copper/apomorphine and iron/apomorphine complexes, respectively. These peaks were not observed in the absence of either epomorphine or copper and iron ions.

Analysis of the stochiometry of these complexes according to Nagano *et al.*[21] indicates a 1:0.9 apomorphine/copper complex (Fig. 7A), and 1.8:1 apomorphine/iron complex (Fig. 7B). Solutions containing apomorphine (0.1 mM) with Cu (II) (0.1 mM) or apomorphine (0.1 mM) with (Fe(III) $(NTA)_2)^{3-}$ (0.05 mM), that were exposed to room air for 45 min, acquired a blue color due to apomorphine oxidation[24] and probably represent low concentration of its radical intermediate. But the wave length and amplitude of the specific peaks respective to apomorphine-Fe(III) and apomorphine-Cu(II) complexes were not affected.

The Effect of Apomorphine on Copper-catalyzed Ascorbate Oxidation

Ascorbate concentration remained almost unchanged during 2 h of incubation at 37°C [without Cu(II)], and the apparent first order rate constant (k) was calculated to be less than $1 \times 10^{-5}\,min^{-1}$ (Table III). Addition to Cu(II) ions to the reaction mixture resulted in a marked enhancement of ascorbate oxidation ($k = 1.4\,min^{-3}$). Apomorphine attenuated ascorbate oxidation rate by 14-fold ($k = 0.1\,min^{-3}$, Table III).

DISCUSSION

The present study demonstrates the cardioprotective effects of low apomorphine concentration. Apomorphine (2 μM) perfused during 20 min before the onset of global ischemia or throughout the first

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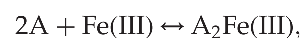
20 min of the reperfusion improves recovery of hemodynamic function (Tables I and II). Furthermore, apomorphine introduced during reperfusion, causes lower protein release at the early phase of reperfusion (Fig. 4). We conclude that this effect is a result of a lower oxidative stress, which decreases the degree of injury to these hearts. Indeed, it has been shown, that protein release from the heart to the coronary fluid can serve as a *bona fide* indicator of tissue injury.[20]

In vitro experiments were carried out in order to understand the mechanism of the beneficial effects of apomorphine on hearts exposed to ischemia–reperfusion. Specific absorbance peaks were observed upon interaction of apomorphine and Cu(II) at 339 nm, and upon interaction of apomorphine and $(Fe(III)(NTA)_2)^{3-}$ at 321 nm. It is suggested that these peaks correspond to newly formed complexes of apomorphine–Cu(II) with an approximate stoichiometry of 1:1, and apomorphine–Fe(III) with an approximate stoichiometry of 2:1, respectively. It is noteworthy that instrument sensitivity limitation did not allow the use of lower apomorphine concentrations (and those of copper and iron) in our *in vitro* experiments.

Apomorphine–Fe(III) complex was obtained by mixing apomorphine with $(Fe(III)(NTA)_2)^{3-}$ at a ratio of 2:1. The stability constant of the complex $(Fe(III)(NTA)_2)^{3-}$ at 20°C is 10^{16}.[25] Thus, the stability constant of apomorphine–Fe(III) complex must be several orders of magnitude higher than that of Fe-NTA. Using this stability constant ($K = 10^{16}$) and the reaction of association/dissociation:

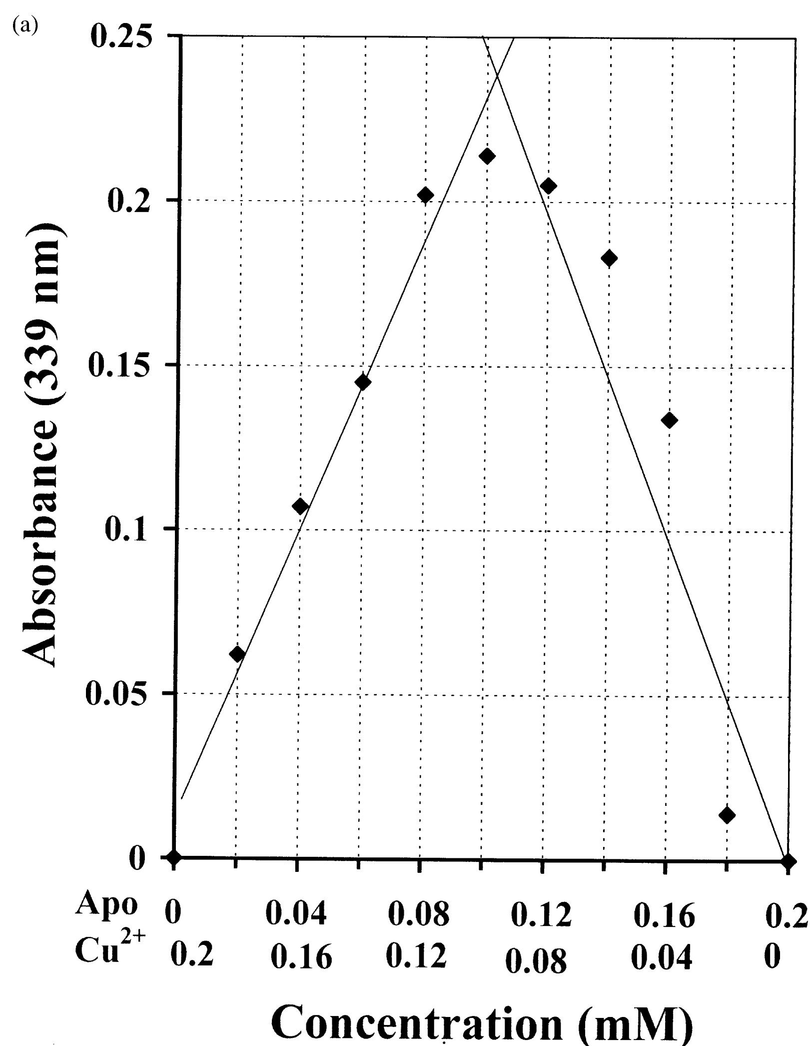


$$2A + Fe(III) \leftrightarrow A_2Fe(III),$$

where A = apomorphine, we calculated the theoretical ratio between the complex apomorphine–Fe(III) to its reactants in the perfused heart. Thus,

$$K = 10^{16} = [A_2Fe(III)]/[A]^2 \times [Fe(III)]$$

Taking into account that the concentration of apomorphine in the perfused heart was 2 μM and that of non-protein-bound iron in the heart at the same order of magnitude,[4] we calculated that more



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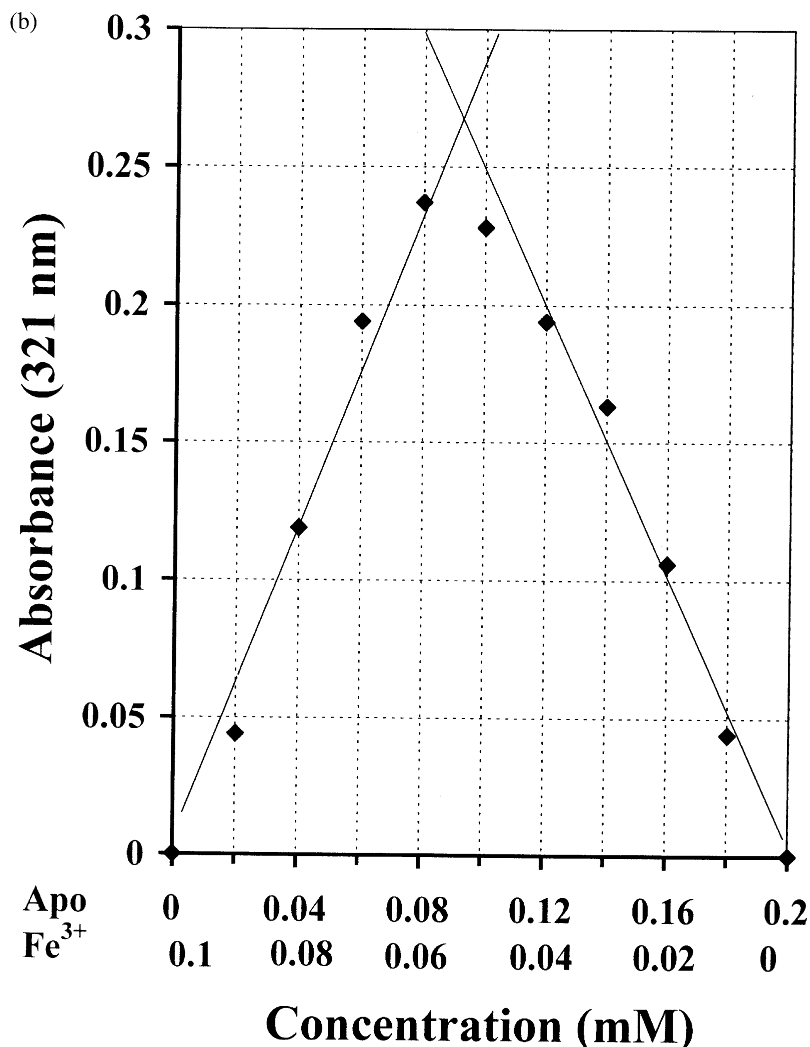

FIGURE 7 The absorbance of the complexes of apomorphine with: (A)—Cu (II) at 339 nm and (B)—Fe (III) at 321 nm, at various [Cu]/[apomorphine] and [Fe]/[apomorphine] ratio.

than 97% of the perfused apomorphine is in the form of apomorphine–Fe(III) complex. Thus, essentially each Fe(III) that is released from its internal pools during ischemia/reperfusion will bind to apomorphine molecules present during early reperfusion. Our *in vitro* experiments indicate that the chelate is relatively stable when exposed to ambient air. Apomorphine–Cu^{2+} chelate is also stable when exposed to ambient oxygen.

Autooxidation of ascorbate can serve as a test for the presence of redox-active metals in buffers.[22] Redox activity of transition metals is an obligatory condition of its participation in hydroxyl radical production by Heber–Weiss reaction.[1] In the present study apomorphine decrease the *in vitro* Cu(II)-induced ascorbate oxidation. Thus, apomorphine binding to transition metals renders these metals redox inactive.

TABLE III Apparent first order rate constant (*k*) for the copper-catalyzed ascorbate oxidation in the absence or presence of apomorphine

System	Ascorbate (5 mM)	O_2 (ambient air)	Cu (50 μM)	Apomorphine (50 μM)	$K \times 10^{-3}$ (min^{-1})
1	+	+	−	−	<0.01
2	+	+	+	−	1.4
3	+	+	+	+	0.1

*A 1:1 ratio of Cu/apomorphine was chosen since this was found to be the ratio in Cu(II)–apomorphine complex (see Fig. 7).

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Copper forms planar, tetrahedral or octahedral complexes with at least four ligands associated with each Cu(II).[26] Figure 7A indicates an apomorphine/Cu(II) ratio of about 1:1. Thus, other ions, such as Tris from the buffer, may participate in this specific complex.

At neutral and alkaline pH ranges the redox potential for iron in aqueous solutions favors the ferric state Fe(III). In the ferric state iron slowly forms large polynuclear complexes with hydroxide ions, water and other anions that may be present.[27] In hemoglobin the iron is conjugated to four nitrogens of the protoporphyrin and two imidazole nitrogens contained in two histidine residues within the potein.[27] Thus, the apomorphine/Fe(III) ratio of about 2:1 in our study (Fig. 7B), may imply that other ions participate in this complex.

Copper and iron are essential components of proteins and are involved in the catalytic function of numerous enzymes.[28] However, upon leakage from stores and release from macromolecular structures, these ions may become involved in deleterious tissue processes.[29–34] Low molecular-weight complexes of copper and iron, which are present in tissues, can serve as redox-active centers for repetitive production of free radical reactions. These can cause damage in the vicinity of the metal-binding site.[35] Recently, direct evidence for substantial iron and copper mobilization into the coronary flow immediately after prolonged ischemia has been reported. The levels of iron and copper in the post-ischemic coronary flow correlates well with the loss of cardiac function following global ischemia of varying duration.[36] In the present study apomorphine at a concentration of 2 μM caused optimal protection of the heart following ischemia–reperfusion. It seems to be quite sufficient for chelation of mobilized iron and copper ions and prevention of oxygen-derived free radicals formation during reperfusion.

Iron catalyzes lipid peroxidation during reperfusion.[37] It has been demonstrated that apomorphine, containing a catechol group, prevents iron-induced lipid peroxidation.[14,38,39] We presume that this effect also may be associated with the formation of apomorphine–iron complex.

Thus, our study suggests that the cardioprotection mechanism of apomorphine stems from its capability to efficiently chelate the transition metals copper and iron that abolishes their redox activity.

In the non-ischemic perfused heart apomorphine (2 μM) increases coronary flow and improves hemodynamic function. The elevated coronary flow persists at least 30 min after the cessation of apomorphine addition in the perfusate (Fig. 5). The increase of coronary flow in hearts treated with apomorphine was observed also after global ischemia (Figs. 1 and 3). Such physiological effects were found previously for dopamine-like agonists. Low doses of dopamine and D_1–D_2 receptor agonists stimulate mainly D_1-like receptors through adenylate cyclase activation, which in turn, induce coronary vasodilatation and increase cardiac output.[40] Stimulation of D_2-like receptors can also contribute to vasodilatation by inhibition of norepinephrine release[41–43] particularly from cardiac sympathetic nerve endings.[44] Apomorphine can increase the nitric oxide synthase activity by D2 receptor stimulation,[45,46] causing a vasodilation effect and coronary flow increase during reperfusion.[47–49]

This D_1 and D_2 receptors stimulation by apomorphine, leading to hemodynamic function increase, is a late effect and is achieved through a signal transduction pathway. However, the effect of apomorphine on the ischemic and reperfused heart in the present study, is rather an immediate effect, and it probably stems from its direct interaction with the transition metal ions iron and copper.

In conclusion, 2 μM apomorphine perfused for 20 min before global ischemia or during 20 min of reperfusion can considerably improve hemodynamic performance, and reduce myocardial injury in the isolated rat heart. This protective effect of apomorphine may be related to its iron and copper chelation properties, which decrease free radical formation during early reperfusion. Further improvement of hemodynamic function recovery during reperfusion may be achieved by the dopaminergic activity of apomorphine. More studies are needed in order to fully assess the use of this unique substance for protecting the human heart during ischemia and reperfusion.

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