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BENEFICIAL EFFECT OF TAN-SHEN, AN EXTRACT FROM THE ROOT OF SALVIA, ON POST-HYPOXIC RECOVERY OF CARDIAC CONTRACTILE FORCE

SATOSHI TAKEO,* KOUICHI TANONAKA, KATSUMI HIRAI,† KAZUKO KAWAGUCHI,† MIKA OGAWA,† AKIRA YAGI† and KOUICHI FUJIMOTO†

Department of Pharmacology, Tokyo College of Pharmacy, Hachioji, Tokyo 192-03 and † Faculty of Pharmaceutical Sciences, Fukuyama University, Fukuyama 729-02, Japan

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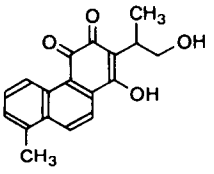
Abstract—The present study was undertaken to elucidate the possible effects of tanshinone VI, one of the extracts from the root of Salvia, on post-hypoxic recovery of cardiac contractile force. For this purpose, rat hearts were perfused for 45 min under reoxygenated conditions following 20-min hypoxic perfusion, and changes in tissue high-energy phosphates and calcium contents, and release of ATP metabolites and creatine kinase were examined. Post-hypoxic recovery of cardiac contractile force was augmented when hearts were treated with 42 nM tanshinone VI during hypoxia. This beneficial recovery was accompanied by enhanced restoration of myocardial high-energy phosphates, depression of hypoxia- and reoxygenation-induced increase in tissue calcium content, and suppression of release of ATP metabolites such as adenosine, inosine and hypoxanthine from the perfused heart. The results suggest that tanshinone VI is beneficial for the recovery of cardiac contractility after a certain period of oxygen-deficiency, possibly through mechanisms involving improvement of myocardial energy production upon oxygen-replenishment and/or inhibition of calcium accumulation in the cardiac cell.

Tan-Shen, an extract from the root of *Salvia miltiorrhiza*, has long been used in Southeast Asia as a therapeutic modality for heart diseases such as angina pectoris and myocardial infarction [1, 2]. The extract has been conceived to improve systemic circulation, in particular, coronary circulation [3, 4]. However, information concerning the effect of the agent on ischemic heart disease is vague, and the exact mechanism for the therapeutic basis is poorly understood. In a previous report from our laboratories, we showed a beneficial effect of some of the extracts on post-hypoxic recovery of cardiac contractile force [5]. The present study was undertaken to characterize further the effect of Tan-Shen on myocardial function and metabolism during oxygen-deficiency and subsequent oxygen-replenishment. Among the extracts from the root of Salvia, we preliminarily confirmed tanshinone VI (tanshinone), whose chemical structure is shown in Fig. 1, to be most effective in the post-hypoxic recovery of cardiac contractile force in perfused rat hearts. Therefore, the present study focused on the effects of this compound on cardiac function and metabolism in the hypoxic and post-hypoxic heart.

METHODS

Perfusion of isolated rat hearts. Male Wistar rats, weighing 200–240 g, were used in the present experiment. The perfusion technique employed was essentially similar to that used for perfusion of rabbit hearts with minor modification [6]. Briefly, rat hearts were quickly isolated after decapitation, and perfused at 37° with Krebs–Henseleit solution, equilibrated with a gas mixture of 95% O_2 + 5% CO_2, at a constant flow rate of 8 mL/min in a non-recirculating Langendorff technique. The heart was preloaded with an initial resting tension of 1.5 g and paced at a rate of 272 beats/min under the stimulating conditions of 0.4 V of strength and 1 msec of duration using an electronic stimulator (Nihonkohden SEN-3201, Tokyo, Japan). The pH of the perfusing solution was monitored continuously with a pH meter (Hitachi-Horiba M-7, Kyoto, Japan) and maintained at 7.40 to 7.42 during perfusion, if necessary, by adding a small amount of solid $NaHCO_3$. After ensuring the equilibration for 30 min, the heart was perfused for 20 min with a glucose-free, Krebs–Henseleit solution saturated with a gas mixture of 95% N_2 + 5% CO_2 (hypoxic perfusion). After 20 min hypoxic perfusion, the heart was perfused for 45 min at 37° with a Krebs–Henseleit solution containing glucose, pre-equilibrated with a gas mixture of 95% O_2 + 5% CO_2 (reoxygenated perfusion). The perfusion pressure was monitored through an aortic cannula adjusted just before the aortic valve by means of a pressure transducer (Nihonkohden TP-101T, Tokyo, Japan). The cardiac contractile force was estimated with developed tension monitored through a hook attached to the apex of the heart by a force-displacement transducer (Nihonkohden TB-621T, Tokyo, Japan). Changes in perfusion pressure, contractile force, and resting tension were recorded on a thermal pen-recorder (Nihonkohden WT-647G, Tokyo, Japan). Treatment with tanshinone was performed by injecting 33.3 nmol/mL of tanshinone at an infusion rate of 0.1 mL/min. This means that the final concentration of tanshinone was 42 nM. In a preliminary study, the relationship between doses of

* Correspondence: Satoshi Takeo, Ph.D., Department of Pharmacology, Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan.



Tanshinone VI

Fig. 1. Chemical structure of tanshinone VI.

tanshinone and recovery of cardiac contractile force after reoxygenation was examined. The percent recovery of the contractile force after treatment with 0 (control), 4.2, 14, 42, and 139 nM tanshinone was 14.1, 14.3, 20.0, 47.7 and 26.7% of the initial value respectively. We therefore used 42 nM tanshinone for the following experiment. Tanshinone VI was isolated from the extract of the root of Salvia as described previously [5].

Measurement of ATP metabolites and creatine kinase activity of the perfusate. The perfusate eluted from the perfused heart was analyzed. At first, changes in ultraviolet absorbance of the perfusate were measured at 250 nm. This was found to be a rough indicator of the release of adenine nucleotide metabolites from the heart as confirmed in a previous study [7]. Then, creatine kinase activity of the perfusate was assayed according to the method of Bergmeyer *et al.* [8]. The perfusate was further subjected to HPLC (Hitachi 611, Tokyo, Japan) analysis by methods described previously [7]. The perfusate was passed through an ODS-column 15 cm in length and 4.6 mm in diameter (Cosmosyl 5C18, Nacalai Tesque, Kyoto, Japan). Separation was made with a buffer of 0.25 M $(NH_4)H_2PO_4$ and 5% CH_3CN, pH 6.2. The eluate was monitored at 254 nm.

Determination of tissue high-energy phosphates. At an appropriate experimental sequence, the heart was clamped with stainless tongs pre-cooled in liquid nitrogen. The metabolites were extracted with 0.3 M trichloroacetic acid + 0.25 mM disodium ethylenediamine tetraacetate under cooling with liquid nitrogen. The extract was centrifuged at 1000 *g* for 20 min. The resulting supernatant solution was neutralized with 2.5 M K_2CO_3 and employed as a sample for determination of tissue myocardial high-energy phosphates. Measurement of myocardial adenosine triphosphate (ATP) was performed according to the method of Bücher [9]. For the determination of myocardial creatine phosphate (CP), the converting reaction of CP to ATP was measured according to the method of Lowry and Passonneau [10]. The details of the determination were described previously [6, 7].

Determination of tissue calcium content. In another set of experiments, hearts were perfused in the same manner as above, and the tissue calcium content was determined as described previously [6]. After perfusion, the coronary arteries were washed with a cold solution of 10 mL of 320 mM sucrose–20 mM Tris/HCl, pH 7.4. Approximately 100 mg of the myocardium was cut into eight pieces, weighed, and dried at 120° for 15 hr. The extraction of tissue calcium was performed according to the method of Grochowski *et al.* [11]. The calcium concentration was determined by an atomic absorption method using an atomic absorption spectrometer (AA 645, Shimazu, Kyoto, Japan).

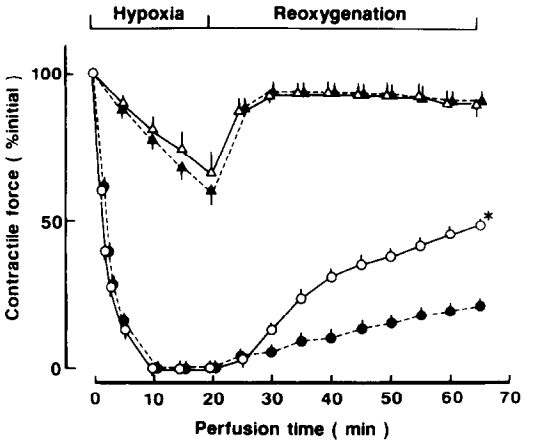


Fig. 2. Percent changes in cardiac contractile force of perfused rat hearts during normoxic, hypoxic and reoxygenated perfusion. Key: (○) changes of the heart subjected to hypoxia and reoxygenation with tanshinone treatment, (●) changes of the heart subjected to hypoxia and reoxygenation without treatment, (△) changes of the heart subjected to normoxia with tanshinone treatment, and (▲) changes of heart subjected to normoxia without treatment. The initial value for the cardiac contractile force was 3.3 ± 0.1 g with initial resting tension of 1.5 g (N = 40). Each value is the mean ± SE of 10 experiments. Statistical significance was calculated using values at 20 min hypoxia and subsequent 45 min reoxygenation. (*) Significantly different from the value without tanshinone treatment ($P < 0.05$).

Statistics. Results are expressed as means ± SE. Statistical significance was calculated using Student's *t*-test for comparison between tanshinone-treated and non-treated groups. Results with a probability of 5% or less were considered to be statistically significant ($P < 0.05$).

RESULTS

Changes in contractile force, perfusion pressure and resting tension of hearts subjected to hypoxia and subsequent reoxygenation. Rat hearts were preperfused for 30 min at 37° with Krebs–Henseleit solution containing glucose. Cardiac contractile force preloaded with an initial resting tension was 3.3 ± 0.1 g and the perfusion pressure, 72 ± 5 mm Hg (N = 40) when monitored at 0 min perfusion (after 30 min preperfusion). The time courses of changes in cardiac contractile force, perfusion pressure and resting tension under normoxic or hypoxic and reoxygenated conditions are shown in Figs. 2, 3 and 4 respectively. When the hearts were subjected to hypoxic perfusion, an immediate decline in cardiac contractile force and a decrease in perfusion pressure were observed. After 5 min of the hypoxic perfusion, cardiac contractile force reached to less than 5% of the initial tension and the perfusion pressure maximally declined to about 38 mm Hg.

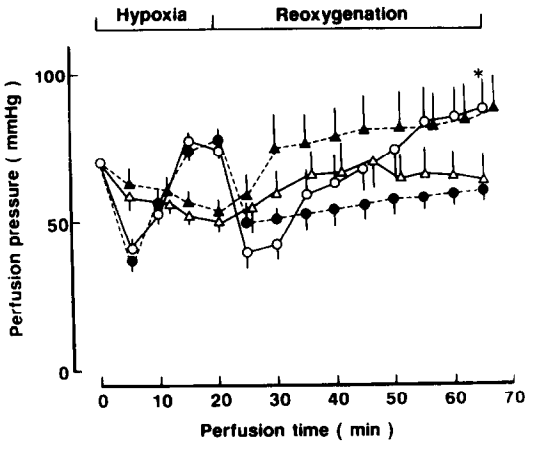


Fig. 3. Changes in perfusion pressure of perfused rat hearts during normoxic, hypoxic and reoxygenated perfusion. The initial perfusion pressure was 72 ± 5 mm Hg (N = 40). The key for the symbols used in this figure is the same as that given in the legend of Fig. 2. Each value is the mean ± SE of 10 experiments. Statistical significance was calculated using values at 20 min hypoxia and subsequent 45 min reoxygenation. (*) Significantly different from the value without tanshinone treatment ($P < 0.05$).

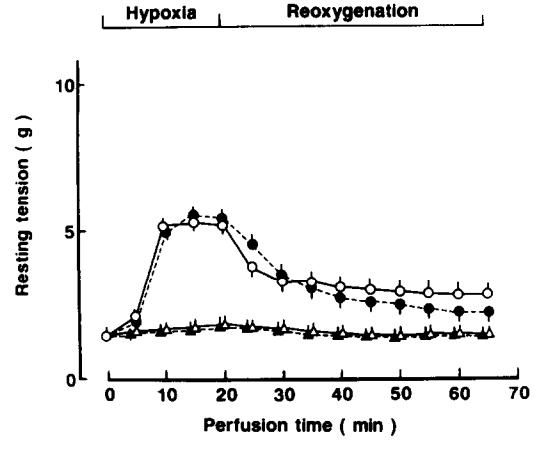


Fig. 4. Changes in resting tension of perfused rat hearts during normoxic, hypoxic and reoxygenated perfusion. The initial resting tension was 1.5 g. The key for the symbols used in this figure is the same as that given in the legend of Fig. 2. Each value is the mean ± SE of 10 experiments. Statistical significance was calculated using values at 20 min hypoxia and subsequent 45 min reoxygenation. There was no significant difference in the resting tension between the two groups ($P > 0.05$).

The resting tension began to rise after 5 min of the hypoxic perfusion. At 20 min of the hypoxic perfusion, no cardiac contractile force was generated, and the perfusion pressure returned toward the initial level. The peak rise in resting tension was seen after 15–20 min of the hypoxic perfusion. When the hearts were reoxygenated following 20 min hypoxic perfusion, a gradual recovery of the contractile force and a steep decline in resting tension were seen. At 45 min reoxygenation, the contractile force was 21 ± 2% of the initial value and the resting tension, 2.80 ± 0.25 g. The perfusion pressure was reduced immediately upon reoxygenation and reached 51 mm Hg at 5 min of reoxygenation; then the pressure gradually rose during reoxygenation. The perfusion pressure reached 61 ± 3 mm Hg at 45 min reoxygenation.

When the hearts were treated with 42 nM tanshinone during hypoxia, no appreciable effects on hypoxia-induced changes of cardiac contractile force, perfusion pressure and resting tension of the perfused hearts were seen. It should be noted that a hypoxia-induced decline in cardiac contractile force during the first 10 min of hypoxic perfusion was seen regardless of treatment with tanshinone. However, the cardiac contractile force was significantly recovered upon reoxygenation; the contractile force at 45 min reoxygenation was 48 ± 2% of the initial, which was about double that in non-treated groups. There was a significant difference in the perfusion pressure at 45 min reoxygenation with and without tanshinone treatment; the perfusion pressure was higher in rat hearts treated with tanshinone than in those without treatment. Treatment of hypoxic hearts with tanshinone did not produce any significant influence on reoxygenation-induced changes in resting tension.

The cardiac contractile force of hearts under normoxic perfusion was decreased slightly during the first 20 min due to a lack of glucose in the perfusing solution, but returned to 90% of the initial value upon 45 min reoxygenation. These changes were not modified by treatment with tanshinone. Normoxic perfusion also did not produce any significant changes in resting tension regardless of treatment with tanshinone. A significantly higher level of the perfusion pressure of the normoxic heart was seen during subsequent 45 min normoxic perfusion following 20 min glucose-free, normoxic perfusion when the heart had been treated with tanshinone during the first 20 min normoxia.

Changes in UV absorbance of the perfusate during hypoxia and reoxygenation. The UV absorbance of the perfusate collected by timed collection was monitored at 250 nm (Fig. 5). This has been proven in a previous study to be a rough indicator of release of adenine nucleotide metabolites from the perfused heart [7]. A marked increase in the UV absorbance of the perfusate was seen immediately after the onset of hypoxia. The peak value for the increase was observed about 12 min after the onset of hypoxia. Then the absorbance gradually subsided up to 20 min of hypoxia. The absorbance was increased further when hearts were reoxygenated during the first 1 to 3 min of reoxygenation. A steep decline in absorbance was seen after 4 min of reoxygenation.

When hearts were treated with tanshinone, the hypoxia-induced increase in the absorbance was suppressed markedly during hypoxia and subsequent reoxygenation. It should be noted that no appreciable changes in the UV absorbance were observed in the perfusate from normoxic hearts regardless of treatment with tanshinone.

HPLC analysis of the perfusate from perfused hearts. To elucidate substances responsible for the

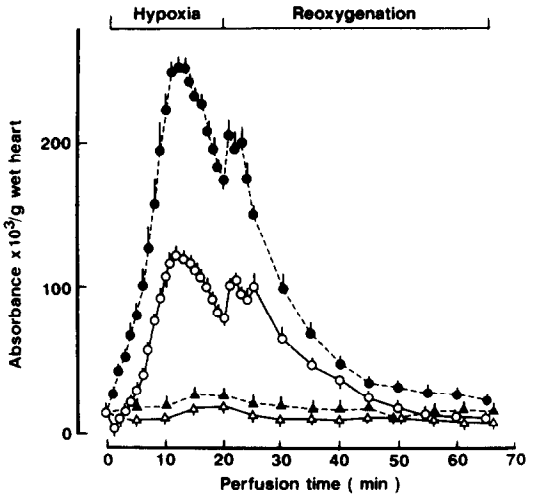


Fig. 5. Changes in ultraviolet absorbance of the perfusate collected by timed collection during normoxic, hypoxic and reoxygenated perfusion. The key for the symbols used in this figure is the same as that given in the legend of Fig. 2. Each value is the mean ± SE of 10 experiments.

increase in the UV absorbance, the perfusate was subjected to HPLC analysis. In previous studies [6, 7], we observed that this increase in UV absorbance was attributed to adenine nucleotide metabolites such as adenosine, inosine and hypoxanthine. As shown in Table 1, the sum of hypoxanthine, inosine and adenosine content in the perfusate was less than 70 nmol/g wet tissue during 20 min normoxic perfusion and less than 90 nmol/g wet tissue during subsequent 45 min normoxic perfusion. Treatment of the normoxic heart with tanshinone did not elicit any significant effect on adenine nucleotide metabolite concentration in the perfusate. Hypoxic perfusion of hearts resulted in a marked increase of the adenine nucleotide metabolites in the perfusate; in particular, inosine was increased most prominently. Subsequent reoxygenation of the heart also induced a marked increase in adenine nucleotide metabolites in the perfusate.

When the heart was treated with tanshinone during the hypoxic period, the release of hypoxanthine, inosine and adenosine was suppressed significantly during hypoxia. The release of adenine nucleotide metabolites was also reduced significantly during subsequent reoxygenation in rat hearts treated with tanshinone during hypoxia.

Creatine kinase (CK) activity of the perfusate. Changes in CK activity of the perfusate are shown in Table 2. CK activity of the perfusate a 0 min perfusion was 55 ± 9 nmol NADPH/g wet heart. There was no appreciable changes in the release of CK from hearts perfused under normoxic conditions regardless of treatment with tanshinone. Hypoxic perfusion produced an increase in CK activity of the perfusate during hypoxia and subsequent reoxygenation; the activity was increased about 3.5-fold at 20 min hypoxia as well as at 45 min reoxygenation. The release of CK during the hypoxic and reoxygenated perfusion tended to be suppressed by treatment with tanshinone but not significantly.

Table 1. Adenine nucleotide metabolite concentrations of the perfusate eluted from hearts perfused under hypoxic and subsequent reoxygenated conditions*

	20 min Perfusion				45 min Perfusion				65 min Perfusion
	Hypoxanthine	Inosine	Adenosine	Total	Hypoxanthine	Inosine	Adenosine	Total	Total
Hypoxia + reoxygenation									
Without tanshinone	239 ± 45	722 ± 154	405 ± 58	1365 ± 232†	376 ± 86	742 ± 125	86 ± 36	1225 ± 151†	2590 ± 307†
With tanshinone	134 ± 17	449 ± 29	230 ± 35	813 ± 62†‡	191 ± 62	372 ± 62	42 ± 23	605 ± 81†‡	1418 ± 128†‡
Normoxia									
Without tanshinone	58 ± 11	ND	ND	58 ± 11	85 ± 6	ND	ND	85 ± 6	143 ± 13
With tanshinone	15 ± 9	46 ± 25	9 ± 8	70 ± 21	65 ± 34	ND	ND	65 ± 34	135 ± 32

* Values are expressed as nmol/g wet heart. Each value is the mean ± SE of 5 experiments. ND = non-detectable.
† Significantly different from the corresponding control group ($P < 0.05$).
‡ Significantly different from the value without tanshinone ($P < 0.05$).

Table 2. Creatine kinase activity of the perfusate eluted from hearts perfused under normoxic, hypoxic and reoxygenated conditions

	Creatine kinase activity (nmol NADPH/g wet heart)	
	At 20 min perfusion	At 65 min perfusion
Hypoxia + reoxygenation		
Without tanshinone	173 ± 12	193 ± 15
With tanshinone	144 ± 12	171 ± 12
Normoxia		
Without tanshinone	45 ± 11	56 ± 14
With tanshinone	51 ± 28	36 ± 21

Each value is the mean ± SE of 5 experiments.

Table 3. Myocardial high-energy phosphates during normoxia, hypoxia and subsequent reoxygenation*

	20 min Perfusion			65 min Perfusion		
	ATP	CP	ATP + CP	ATP	CP	ATP + CP
Hypoxia + reoxygenation						
Without tanshinone	4.89 ± 0.37	5.30 ± 0.51	10.19 ± 0.18	10.55 ± 0.64	20.77 ± 0.72	31.32 ± 1.30
With tanshinone	4.77 ± 0.05	12.06 ± 1.07†	16.82 ± 1.06†	19.68 ± 0.37†	25.54 ± 1.52†	45.23 ± 1.71†
Normoxia						
Without tanshinone	17.15 ± 2.41	29.31 ± 1.88	46.49 ± 1.90	20.52 ± 1.01	26.59 ± 1.79	47.11 ± 2.29
With tanshinone	20.95 ± 1.28	24.10 ± 1.38	45.05 ± 1.04	22.14 ± 0.84	35.33 ± 3.38†	57.47 ± 3.92†

* Values are expressed as μmol/g dry tissue. Each value is the mean ± SE of 5 experiments. The initial values of ATP, CP and ATP + CP contents (at 0 min perfusion) were 23.73 ± 0.96, 37.09 ± 3.68 and 60.82 ± 3.99 μmol/g dry tissue respectively.

† Significantly different from the group without tanshinone ($P < 0.05$).

Tissue high-energy phosphates. Myocardial ATP and CP were determined at 0, 20 min and subsequent 45 min perfusion, and the results are shown in Table 3. Hypoxia induced a marked reduction of the total high-energy phosphate. Subsequent 45 min reoxygenation recovered the levels to about half of the initial values. Treatment of hypoxic hearts with tanshinone resulted in a significant preservation of tissue high-energy phosphates during hypoxia and a significant restoration of the high-energy phosphates upon subsequent reoxygenation. Normoxic perfusion of rat hearts during the first 20 min perfusion also reduced tissue high-energy phosphates due to a lack of glucose in the perfusing medium; particularly, reduction of CP was prominent. Reperfusion of the normoxic heart for 45 min with the buffer containing glucose resulted in a recovery of ATP and CP, but not to a level similar to the initial values. It should be noted that the high-energy phosphate level of normoxic hearts treated with tanshinone was similar to the initial value after 45 min reperfusion.

Tissue calcium contents after hypoxia and reoxygenation. Tissue calcium contents of the heart perfused at 20 min and subsequent 45 min perfusion were determined, and the results are shown in Fig. 6. Control calcium content was 2.31 ± 0.1 μmol/g dry tissue ($N = 6$). Hypoxia induced a significant increase in the tissue calcium content, and reoxygenation also produced a further increase in the calcium content. A significant suppression in the tissue calcium content was seen in hypoxic hearts after treatment with tanshinone. Such suppression was also observed in the reoxygenated heart with this treatment, but to a lesser extent. There were no

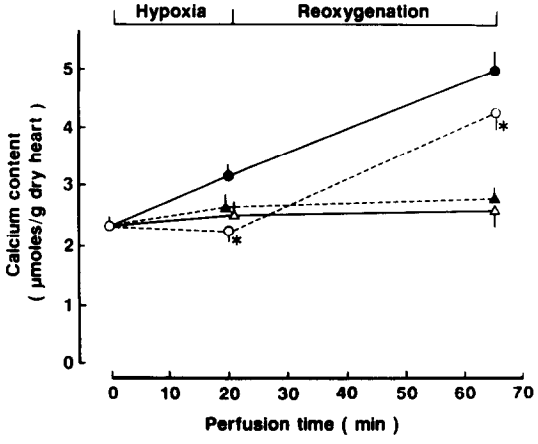


Fig. 6. Tissue calcium content of rat hearts at 20 min normoxia or hypoxia and subsequent 45 min normoxia or reoxygenation. The key for the symbols used in this figure is the same as that given in the legend of Fig. 2. Each value is the mean ± SE of 5 experiments. (*) Significantly different from the values of non-treated group ($P < 0.05$).

appreciable differences in the tissue calcium content of normoxic hearts regardless of tanshinone treatment.

DISCUSSION

In the present study, we have shown that treatment of hypoxic hearts with tanshinone resulted in a significant recovery of cardiac contractile force after a period of 20 min of hypoxia. This was associated with preservation of myocardial high-energy phosphate levels at 20 min hypoxia and restoration of these compounds at 45 min reoxygenation, relative to those without tanshinone treatment. It is well recognized that calcium antagonists may exert their cardioprotective effect through negative inotropic action by which they are capable of preserving myocardial energy levels [12–15]. This is not unlikely in the present experiment because cardiac contractility in tanshinone-treated hearts was not observed to be depressed significantly during hypoxic perfusion as compared with that of hearts without treatment. However, we observed a significantly high level of myocardial high-energy phosphates in rat hearts at 20 min hypoxia, suggesting an energy-preserving effect of this agent during hypoxic perfusion, which is independent of cardiac contractility. At present, it is difficult to elucidate a mechanism to which this phenomenon is attributable.

It should be noted that restoration of the myocardial high-energy phosphate levels during reoxygenation was augmented greatly in rat hearts treated with tanshinone. There is evidence that recovery of cardiac contractile force after a certain period of oxygen-deficiency is associated with an appreciable restoration of myocardial high-energy phosphates [16, 17]. In contrast, Neely and Grotyohann [18] have claimed that recovery of cardiac contractile force is independent of tissue high-energy phosphate levels and proposed an important contribution of tissue lactate to an insufficient post-ischemic recovery of cardiac contractility after oxygen deficiency. However, this is not pertinent to a mechanism responsible for sustained cardiac dysfunction after hypoxia in the present experiment, because the hypoxic hearts in the present experiment, unlike ischemic hearts, were perfused continuously with the buffer whose pH was maintained at 7.40 to 7.42 throughout the experiment. This could eliminate easily removable metabolites from the heart. Thus, it is conceivable that accumulation of metabolites within the myocardium occurs during perfusion to a minimal degree, if any. Although a cause-and-effect relationship between myocardial high-energy phosphate levels and post-hypoxic or post-ischemic recovery of cardiac contractile force is still controversial, recovery of myocardial high-energy phosphate levels is one of the possible factors contributable to a better recovery of cardiac contractile force after oxygen deficiency.

In the previous study, we have shown that calcium antagonists, such as diltiazem and verapamil, may exert a beneficial effect on post-hypoxic recovery of cardiac contractile force, possibly through the mechanism by which hypoxia-induced loss of adenine nucleotide metabolites is prevented [6]. In the present study there was an appreciable suppression of the release of adenine nucleotide metabolites from the perfused heart when hearts were treated with tanshinone. Since adenine nucleotide metabolites are possible substrates for the salvage synthesis of ATP, it is possible that a suppression of release of adenine nucleotide metabolites is beneficial for salvage synthesis of ATP during post-hypoxic recovery. Thus, preservation of the metabolites in the myocardium may be one of the possible mechanisms for beneficial post-hypoxic recovery of cardiac contractile force. This notion is supported by the hypothesis of Ford and Rovetto [19] that prevention of nucleoside loss during ischemia would provide more substrate for ATP production after ischemia. The preservation of these metabolites in the myocardium has also been observed in rabbit hearts which showed a beneficial recovery of cardiac contractile force after reoxygenation when treated with α-adrenoceptor blocking agents [20, 21] and local anesthetics [22].

It is well recognized that calcium overload in the cardiac tissue has been shown to occur when pathophysiological interventions induce an increase in the cell membrane permeability [23–25]. The present experiment has shown an increase in tissue calcium content during hypoxia and subsequent reoxygenation and prevention of calcium overload in the myocardium by treatment with tanshinone. Since calcium overload induces various kinds of deterioration in the cardiac tissue such as exhaustion of myocardial energy [26], impairment in the mitochondrial function [27–29], change in cardiac subcellular membrane activity [30] and damage to structural integrity [31, 32], suppression of the calcium overload is positively beneficial for protection of myocardial cells exposed to oxygen deficiency, which may lead to a better recovery of cardiac contractile force after hypoxia.

In the present study, a significant release of CK was seen during hypoxia, whereas a further increase in the release of CK from the perfused rat heart was not observed. This is, in part, different from the observation in rabbit hearts under the same conditions as above [20–22]. This suggests that rabbit hearts are much more sensitive to oxygen deficiency than rat hearts. A failure to demonstrate prevention of hypoxia- and reoxygenation-induced increase in the release of CK may represent ineffectiveness of tanshinone on the increase in cell membrane permeability and/or the induction of cardiac cell necrosis under such pathophysiological conditions, since release of CK is recognized to be an indicator of these disturbances [33–35].

As mentioned at the beginning of the paper, little information is available concerning mechanisms responsible for the effectiveness of Tan-Shen on ischemic hearts. The present study suggests two possible mechanisms for the recovery of post-hypoxic cardiac contractility, i.e. enhancement of the myocardial energy store restoration after oxygen deficiency and prevention of hypoxia- and reoxygenation-induced calcium overload in the cardiac tissue. These mechanisms should be explored to determine the pharmacological profile of the extracts from the root of *Salvia miltiorrhiza*.

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