

Regulatory Effects of Chronic Low-dose Morphine on Nitric Oxide Level Along With Baroreflex Sensitivity in Two-kidney One-clip Hypertensive Rats

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Introduction. Opiates are traditionally used for treatment of some acute heart disorders. There are only few reports on the effects of long-term treatment of cardiovascular diseases with morphine. This study aimed to investigate the effects of chronic low-dose morphine use on the cardiovascular system in two-kidney one-clip (2K1C) hypertensive rats.

Materials and Methods. Male Wistar rats were divided into two groups as the sham and 2K1C groups and each group was further subdivided into saline and morphine treatment subgroups. Blood pressure, heart rate, plasma rennin activity, serum nitric oxide concentration, and baroreflex sensitivity were measured.

Results. Morphine significantly attenuated systolic blood pressure, diastolic blood pressure, and mean arterial pressure in the 2K1C animals. In addition, morphine decreased plasma rennin activity in the 2K1C group. Serum concentrations of nitric oxide were also decreased, and morphine prevented the reduction of nitric oxide. The baroreflex sensitivity was also improved following morphine administration in the 2K1C group.

Conclusions. According to the results presented in this study, chronic administration of low-dose morphine reduces regulated hypertension in the 2K1C rats, probably via a nitric oxide-dependent pathway.

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INTRODUCTION

Hypertension is considered as a major public health issue. Hypertensive patients are at risk of several cardiovascular complications related to endothelial dysfunction, baroreflex sensitivity (BRS), and rennin-angiotensin-aldosterone system (RAAS) imbalance.^{1,2} Endothelial dysfunction is defined as an imbalance between the production and bioavailability of endothelium-derived relaxing factors.³ Nitric oxide (NO) has been identified as the

most known endothelium-derived relaxing factor. Various physiological stimuli induce NO synthesis from L-arginine in endothelial cells.⁴ Baroreflex sensitivity is amongst the most important nervous regulatory mechanisms by which the blood pressure is regulated.⁵ The arterial baroreflex is a negative-feedback mechanism, essential for maintaining arterial pressure at constant levels. The RAAS inhibitors are one of the key therapeutic targets in patients with hypertension, as an overactive RAAS

is strongly associated with high blood pressure.⁶

Opioids regulate cardiovascular activity, and the strong effects of opioid peptides on blood pressure and heart rate suggest that these peptides may consider as one of the important endogenous substances in central cardiovascular control.^{7,8} Studies demonstrate that prolonged administration of morphine decreases the plasma rennin activity (PRA), increases NO synthesis, improves BRS, and modulates RAAS.⁹⁻¹¹

Most of previous studies have focused on investigating the cardiovascular effects of high dose and acute use of morphine. There exist reports indicating a high dose of morphine has cytotoxic effects on macrophages,¹² vascular endothelium,¹³ mesangial cells, and epithelial cells.¹⁴ However, few studies are available regarding the effect of chronic low dose of morphine on cardiovascular-related changes in hypertensive animals. In the present study, we sought to examine the effects of prolonged low-dose morphine on some cardiovascular related changes (BRS, PRA, and NO) in two-kidney one-clip hypertensive (2K1C) rats.

MATERIAL AND METHODS

Animals

Thirty-two male Wistar rats (weighting of 200 ± 20 g) were enrolled in this study. The rats were housed at a controlled temperature (22°C) and had free access to food and water. They were randomly divided into 4 groups of sham operated (2 groups) and 2K1C induced hypertensive rats (2 groups). Sham operated animals were further allocated into saline and morphine groups. Also, 2K1C hypertensive rats were further allocated into saline and morphine groups ($n = 8$). In the morphine-treated groups, 3 mg/kg of morphine sulphate was injected intraperitoneally for 8 weeks. Saline groups received the same volume of saline according to a similar protocol. The experimental protocols used in this study were approved by the Ethics and Animal Care Committee of Rafsanjan University of Medical Sciences and were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Preparation of Hypertensive Rats

Using the Goldblatt method as describe earlier,² the rats were anesthetized with ketamine

hydrochloride, 60 mg/kg, and xylazine, 7.5 mg/kg, injected intraperitoneally. The left kidneys were exposed via a flank incision and a silver clip with an internal gap of 0.2 mm was set around the renal artery. In the sham group, the same procedure was done without using silver clip. Penicillin G, 25000 U IM, was injected after the surgery. The rats were fed with commercial rat chow (Razi Institute, Iran) and had free access to tap water. After 8 weeks, the animals were anesthetized and direct blood pressure was measured by a catheter which was inserted into the femoral artery. Blood samples were taken for further determination of PRA and NO concentrations.

Measurement of Arterial Pressure and Baroreflex Sensitivity

To measure arterial pressure and BRS, the rats were anesthetized by intraperitoneal urethane injection with a dosage of 150 mg/100 g. In order to infuse phenylephrine and sodium nitroprusside, the femoral vein was cannulated. The femoral artery was also cannulated for recording both blood pressure and heart rate. Since a period of blood pressure, mean arterial pressure (MAP), and heart rate were recording, the injection of phenylephrine and sodium nitroprusside were simultaneously performed to measure BRS. The baroreflex was tested with a pressure dose of phenylephrine (8 µg/kg, intravenous; Sigma Chemical, USA) and a depressor dose of sodium nitroprusside (50 µg/kg, intravenous; Sigma Chemical, USA). The baroreflex was calculated as the ratio of changes in heart rate to the changes in MAP. There was an interval of at least 15 minutes between the infusions to allow the recovery of basal values.²

Measurement of Serum Nitric Oxide Concentration

The serum concentrations of NO were measured by the Griess reagent system (Promega Corporation, Madison, USA). Serum samples were added into wells of a 96-well flat-button enzymatic assay plate and subsequently sulfanilamide solution was added to the samples. Finally, N-1-naphtylethylenediamine dihydrochloride reagent was added. The absorbance was read at 520 nm to 550 nm by a microreader.¹⁵ The samples' NO concentration was determined against nitrite standard curve. The limitation of detection was 2.5 µM.

Assessment of Plasma Renin Activity Assay

The PRA was measured with a kit which was purchased from Diasorin Inc by the use of ^{125}I Angiotensin I generation. Angiotensin I-coated tube radioimmunoassay¹⁶ was performed in two aliquots of the same sample so that one of them was incubated at 37°C for generation while the other was not. The PRA was calculated as nanogram of angiotensin I generated per milliliter per hour (Renctk P2721, Sorin-Biomedica Diagnostic Division RIA kit, Italy). The PRA assay sensitivity was 0.13 ng/ml and intra and interassay coefficients of variation were 7.5 and 7.7%, respectively.

Statistical Analysis

Data were analyzed using the SPSS software (Statistical Package for the Social Sciences, version 11.5, SPSS Inc, Chicago, Ill, USA). Comparisons were made with the unpaired *t* test or the analysis of variance method and the Tukey test as a posttest, where appropriate. The results were presented as mean \pm standard error of mean. Differences were considered significant when the *P* value was less than .05.

RESULTS

Blood Pressure and Heart Rate

Systolic blood pressure, diastolic blood pressure, and MAP were significantly increased in the 2K1C group as compared to the sham operated group ($P < .001$). In the sham operated groups, administration of low-dose morphine significantly downregulated diastolic blood pressure ($P < .01$) and MAP ($P < .05$), while it had no effects on either systolic blood pressure or heart rate as compared to saline. In the 2K1C groups, administration of low-dose morphine significantly decreased systolic blood pressure ($P < .01$), diastolic blood pressure ($P < .05$), and MAP ($P < .01$), as compared with saline (Figures 1 to 3).

Baroreflex Sensitivity

The BRS was significantly decreased in the 2K1C group as compared to the sham operated group ($P < .01$). In the sham operated groups, administration of low-dose morphine significantly upregulated the BRS ($P < .05$), when compared with saline. In the 2K1C groups, the BRS was significantly improved ($P < .05$) by administration of a low-dose morphine (Figure 4).

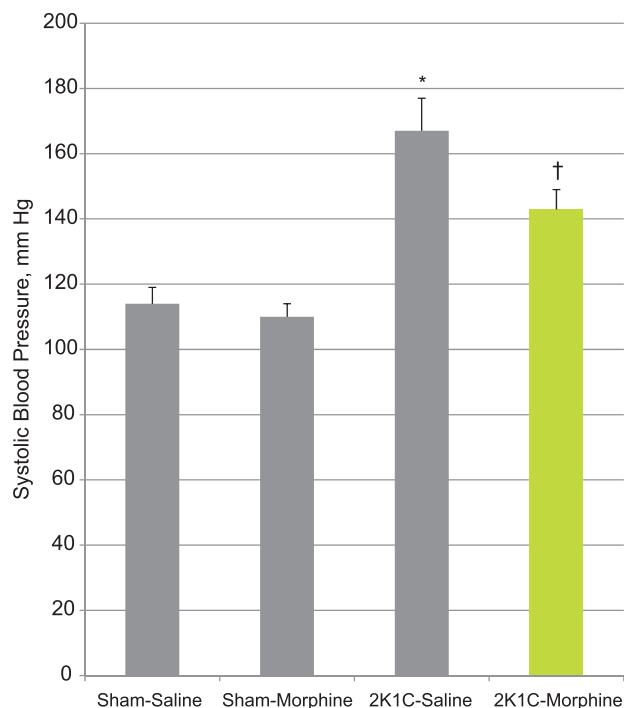


Figure 1. Systolic blood pressure in the sham and two-kidney one-clip (2K1C) rats treated with morphine and saline. * $P < .001$, compared with sham-saline † $P < .01$, compared with 2K1C-saline

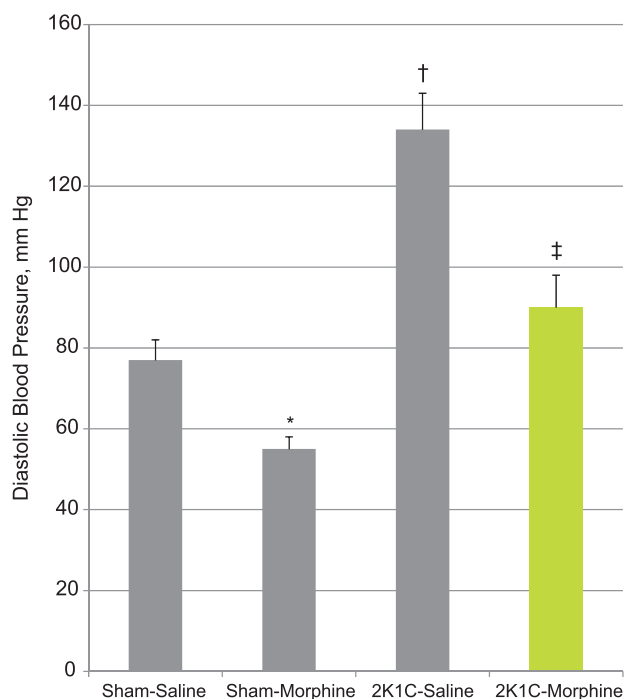


Figure 2. Diastolic blood pressure in the sham and two-kidney one-clip (2K1C) rats treated with morphine and saline. * $P < .01$, compared with sham-saline † $P < .001$, compared with sham-saline ‡ $P < .05$, compared with 2K1C-saline

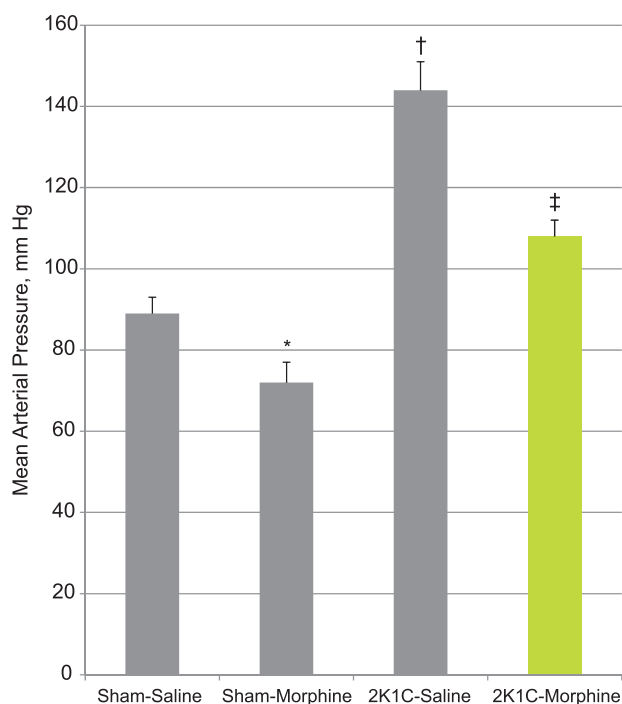


Figure 3. Mean arterial pressure in the sham and two-kidney one-clip (2K1C) rats treated with morphine and saline.
 * $P < .05$, compared with 2K1C-saline
 † $P < .001$, compared with sham-saline
 ‡ $P < .01$, compared with sham-saline

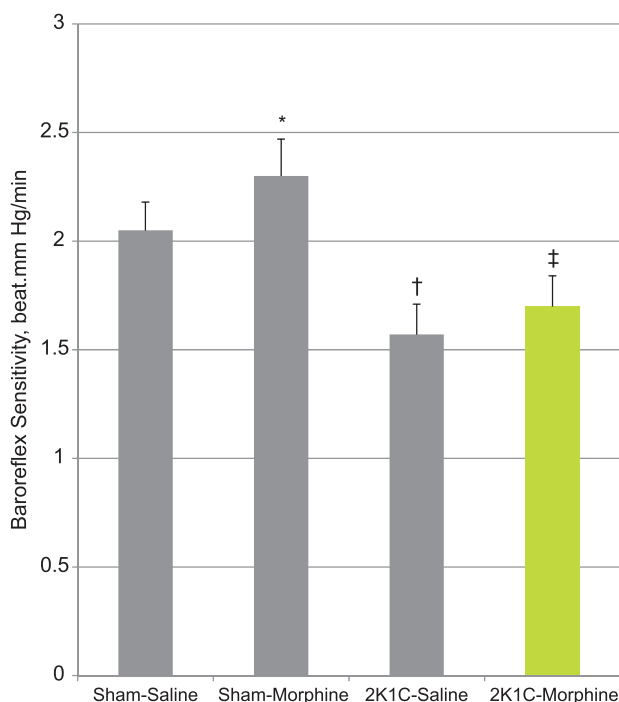


Figure 4. Baroreflex sensitivity in the sham and two-kidney one-clip (2K1C) rats treated with morphine and saline.
 * $P < .05$, compared with sham-saline
 † $P < .01$, compared with sham-saline
 ‡ $P < .05$, compared with 2K1C-saline

Plasma Renin Activity

The PRA levels were significantly higher in the 2K1C groups ($P < .01$), as compared with the sham operated groups. In the sham operated groups, administration of low-dose morphine had not effect on PRA, as compared to the saline-treated group, while in the 2K1C groups, administration of low-dose morphine significantly decreased PRA ($P < .01$; Figure 5).

Serum Nitric Oxide

Serum NO concentrations were significantly decreased in the 2K1C group ($P < .001$), as compared with the sham operated groups. In the sham operated groups, administration of low-dose morphine had no effects on the serum NO concentrations, as compared to the saline-treated group. In the 2K1C groups, administration of low-dose morphine significantly increased serum NO concentrations ($P < .01$), as compared with the 2K1C saline-treated group (Figure 6).

DISCUSSION

The present study was undertaken to evaluate the effects of chronic administration of low-dose morphine on NO concentrations, PRA, and BRS in

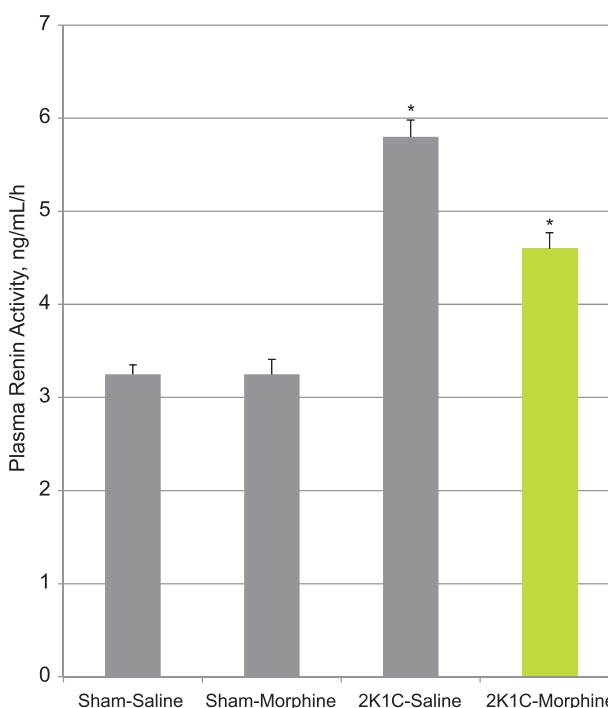


Figure 5. Plasma renin activity levels in the sham and two-kidney one-clip (2K1C) rats treated with morphine and saline.
 * $P < .01$, compared with sham-saline

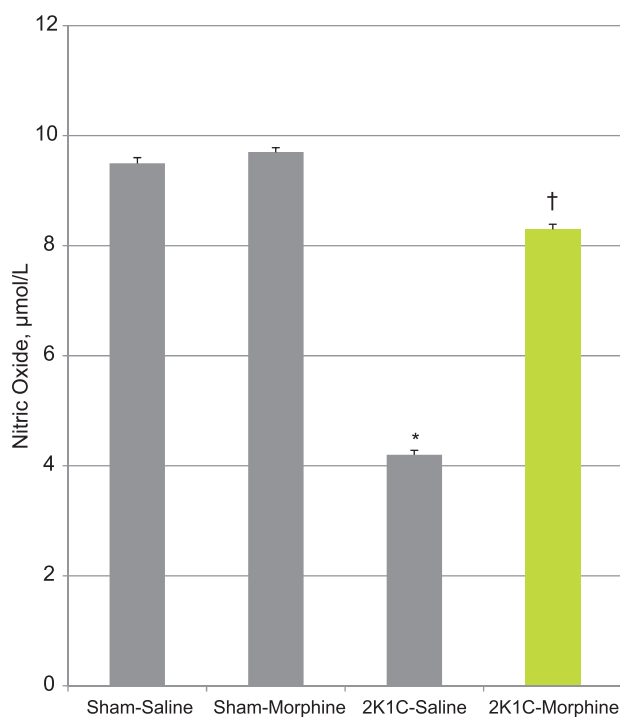


Figure 6. Nitric oxide levels in the sham and two-kidney one-clip (2K1C) rats treated with morphine and saline.

* $P < .01$, compared with sham-saline

† $P < .01$, compared with 2K1C-saline

2K1C hypertensive rats. Our results demonstrated that serum levels of NO and BRS were improved, following long-term administration of morphine, while PRA was decreased. The Goldblatt 2K1C hypertension rat model is a well-established widely employed model for investigating the renal artery stenosis and renovascular hypertension.^{17,18} Previous studies indicate that reduced renal perfusion pressure stimulates rennin production in clipped kidney which may lead to increased blood pressure.¹⁹⁻²¹ In agreement with those studies, we also found that PRA was upregulated in the 2K1C rats.

There exist reports demonstrating that acute morphine downregulated blood pressure and PRA in rats. Jimenez and Fuentes reported that subchronic administration of morphine inhibits the hypertension induced by stresses of isolation in rat.²² It is also well established that acute administration of morphine decreases blood pressure in spontaneously hypertensive rats.²³ It was reported that PRA was elevated in response to naloxone administration in morphine addicted rats.⁹ More recently, we have demonstrated that both acute and chronic morphine treatment downregulated systolic and diastolic blood pressure in rats.²⁴ In the

present study, we showed that chronic low dose of morphine attenuated levels of PRA and diastolic blood pressure in 2K1C hypertensive rats. Taken together with previous reports, one can conclude that both acute and chronic morphine consumption could reduce blood pressure.^{24,25}

Moreover, our results demonstrated that serum NO concentrations were reduced in 2K1C hypertension rats. In agreement with our findings, compelling evidences showed that NO secretion decreased following hypertension in animal models and in clinical studies.^{2,26,27} It has been proposed that disrupted NO pathway in hypertension is probably due to either down regulation of NO production or up regulation of NO degradation. The low NO production in 2K1C hypertensive rats is possibly due to reduced L-arginine concentration or suppression of endothelial NO synthase activity.²⁸ It has also been indicated that reduced renal artery diameter in 2K1C model induces rennin-angiotensin-aldosterone dependent hypertension, thus, a further explanation for reduced NO bioavailability in this model of hypertension might possibly be due to the fact that high angiotensin II level downregulated the NO level via induction of oxidative stress.²⁹ We also found that prolonged low dose morphine reversed the changes in blood pressure and serum NO concentrations close to normal level. Antihypertensive properties have been revealed for endogenous morphine-nitric oxide signaling events.³⁰ Morphine showed to modulate vascular endothelial cells functions via local paracrine-autocrine regulatory pathways. These pathways regulate endogenous expression of NO.³⁰ Furthermore, evidences are in favor of the fact that NO signaling is crucial for opioid receptor-mediated responses in the neurocardiovascular system.² The BRS was attenuated in 2K1C hypertensive rats and chronic low-dose morphine improved BRS in these animals. Previous studies indicated that BRS was lower in hypertensive patients.^{24,31} Recent investigations described the importance of NO in baroreflex function in hypertensive rats.^{32,33}

CONCLUSIONS

Results of present study demonstrated that chronic administration of low-dose morphine could increase serum level of NO and improve BRS, while it decreased PRA in 2K1C rat model of hypertension.

FINANCIAL SUPPORT

This study was financially supported by a grant from Rafsanjan University of Medical Sciences.

CONFLICT OF INTEREST

None declared.

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