# Effect of co-administration of morphine and nicotine on cardiovascular function in two-kidney one clip hypertensive (2K1C) rats

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## Abstract

Cardiovascular morbidity and mortality are potentiated with smoking and hypertension. The aim of this study was to investigate the effects of morphine and nicotine co-administration on cardiovascular function in two-kidney one-clip hypertensive (2K1C) rats. Thirty-two male rats were divided into four groups as follow: Vehicle, morphine, nicotine and nicotine + morphine. All drugs were administered for 8 weeks. Baroreflex sensitivity (BRS), heart rate and blood pressure were measured using a Power Lab data acquisition. Plasma rennin activity (PRA) and serum concentration of nitric oxide (NO) were measured using Elisa method. To induce hypertension, the renal artery of left kidney was clipped for 8 weeks. A significant decrease in systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) was observed in nicotine + morphine group compared to vehicle and nicotine groups ($p<0.05$). Serum concentration of NO was lower in nicotine + morphine group compared to morphine group and significantly higher than nicotine group. The BRS was lower in the nicotine + morphine group compared to other groups. The PRA level was higher in nicotine + morphine compared to morphine group but it was higher than nicotine group. This study demonstrated that prolonged co-consumption of morphine and nicotinedecreased PRA and blood pressure and increased the serum concentration of NO in hypertensive rats. Co-administration of morphine and nicotine decreased BRS in 2k1c hypertensive rats probably via central nervous system.


KEY WORDS: baroreflex sensitivity, blood pressure, nitric oxide, morphine, nicotine

## INTRODUCTION

Today smoking behavior is among it the most accepted preventable causes of death in the United States. Smokers experience a wide range of physiologic side effects that increase the risk of cardiovascular disease (CVD) [1]. It is widely accepted that hypertension and smoking are of potent independent risk factors considered for cardiovascular morbidity and mortality, and concomitant smoking in hypertensive patients may increase the risk of coronary heart disease [2, 3].Prolonged cigarette smoking increased BP, systemic vascular resistance and heart rat [4]. Nitric oxide (NO) is an inevitably important molecule in control of vascular tone, blood flow, peripheral vascular resistance, and systemic blood pressure. Chronic smoking impairs endothelial function via lowering the formation of NO [5]. It is proposed that dysfunction of NO pathway in hypertensive subjects is a consequence rather than a cause of elevated BP [6]. Recent evidences obtained from human based studies showed that smoking of four cigarettes per hour impairs baroreflex sensitivity [7]. Opioid receptors demonstrated to regulate cardiovascular function in both normal and diseased myocardium [8]. It has been reported that systemic administration of morphine decreases MAP in the rat and evokes orthostatic hypotension in the healthy patient [9]. Moreover, morphineinduces NO formation in the endothelium and some other tissues [10]. Recent investigations showed the importance of NO during baroreflex function development in hypertensive rats [11]. Several studies examined the effect of morphine and/or nicotine on cardiovascular system while no studies was undertaken on the effect of co-administration of morphine and nicotine on cardiovascular system. Therefore, in this study we examined the effect of co-administration of morphine and nicotine in the development and/or maintenance hypertension and BRS in hypertensive rats.

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# MATERIAL AND METHODS

In the present study thirty two male Wistar rats weighing 200±20 grams were enrolled. Rats were accommodated at a controlled temperature with free access to food and water. Prior to start of treatment, animals were randomly divided into below 4 different groups: (1): vehicle group (received only saline), (2): morphine group (received 3 mg/kg of morphine), (3): nicotine group (received 0.01 mg/kg of nicotine) and (4): nicotine combined with morphine (received 3 mg/kg of morphine and 0.01 mg/kg of nicotine). All of drugs were administered for a period of 8 weeks.

*Induction of hypertension in rats by 2K1C goldblatt method*

Initially rats were anesthetized using intraperitonealy ketamine hydrochloride (60 mg/kg) and xylazine (7.5 mg/kg). The left kidney was exposed via flank incision and a silver clip with internal gap of 0.2 mm was put around the renal artery. The sham group, were also treated by this procedure except using silver clip. To control the risk of infections, rats received penicillin G (25000 IU/rat) after surgery. Systolic and diastolic blood pressure was measured once a week by a tail-cuff plethysmography under light ether anesthesia. After 8 weeks of treatment, the animals were anesthetized and direct blood pressure was measured by a catheter (PE50) inserted into femoral artery. Blood samples were collected to measure plasma rennin activity (PRA) in further analysis.

*Measurement of Plasma renin activity*

The PRA was measured by a kit using 125 I Angiotensin-I generation. Angiotensin Icoated-tube radioimmunoassay (RIA) wasperformed in two aliquots of the same sample,one incubated at 37 °C for generation and onenon-incubated; PRA was calculated as ngAngiotensin I generated/ml/h (Renctk P2721, Sorin-Biomedica Diagnostic Division RIA kit,Italy). The PRA assay sensitivity was 0.13 ng/ml; intra-and interassay coefficients ofvariations were 7.5 and 7.7%, respectively.

*Measurement of serum NO*

Following 8 weeks of induction of hypertension, blood samples were collected from all animals. The serum NO concentration was measured by Gris reagent system (Promega Corporation, Madison, USA). Serum sample, were added to the wells (96-well flat-button enzymatic assayplate). A sulfanilamide solution was then added to the wells pre-treated with sample and following of additionN-1-naphtylethylenediamine dihydrochloride (NED) to the wells under acidic conditions. Finally, the absorbance were read in 520-550 nm by a microreader [12]. The NO concentration in the samples was determined and compared to NO standard curve. The limit of detection was 2.5 µM nitrite.

*Baroreflex Assessment*

The direct AP and HR were recorded in anesthetized rats. Catheters were connected to a pressure transducer (Maxxim Medical, Athens, TX) coupled to a multichannel recorder by a custom-designed amplifier (University of Iowa, Iowa City, IA). The analog input was converted into a digital signal using a Power Lab data acquisition system (AD Instruments, Mountain View, CA). The MAP was derived electronically using a low-pass filter set at 100 Hz and was calculated using the cyclic mean. Data were acquired at 200 samples/s. HR was determined by measuring the number of heartbeats triggered from the arterial pressure pulse and was calculated online. Once the animal had sufficient time to adapt to the surrounding environment of in the testing chamber, hemodynamic parameters were monitored for 20–40 min to ensure stabilization of MAP and HR. Subsequent to the confirmation of stable hemodynamic parameters, the baseline parameters were continuously recorded for at least 10 min. The baroreflex was tested with a pressor dose of phenylephrine (PE bolus: 8 µg/kg i.v.; Sigma Chemical) and a depressor dose of sodium nitroprusside (SNP bolus: 50 µg/kg i.v.; Sigma Chemical). The baroreflex was calculated as the ratio of HR variations to MAP variations (ΔHR/ΔMAP). There was an interval of at least 15 minutes between the infusions to allow the recovery of basal values [13].

*Statistical analysis*

The results are presented as Mean±S.E.M. Data were analyzed using ANOVA followed by the tukey post-hoc test. Differences were considered statistically significant if $p$ value was less than 0.05.

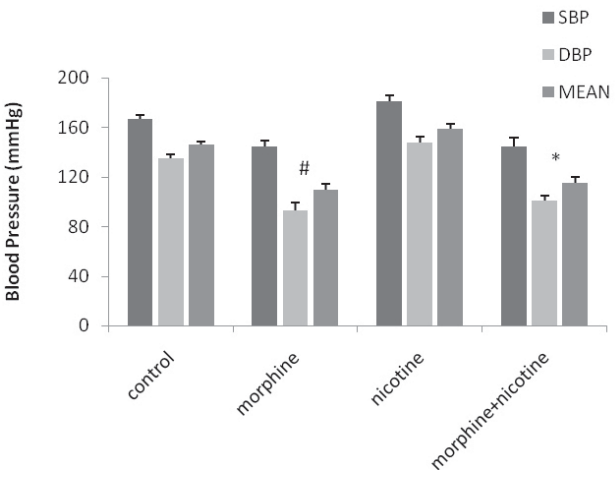


FIGURE 1. Variations in SBP, DBP and MAP in experimental groups. *Significant difference in SBP and DBP between morphine+ nicotine with vehicle and nicotine group (all $p<0.05$)
# Significant difference in SBP and DBP between morphine with vehicle and nicotine group (all $p<0.05$). SBP; systolic blood pressure, DBP; systolic blood pressure, MAP; mean arterial pressure. Data are shown as mean ± S.E.M

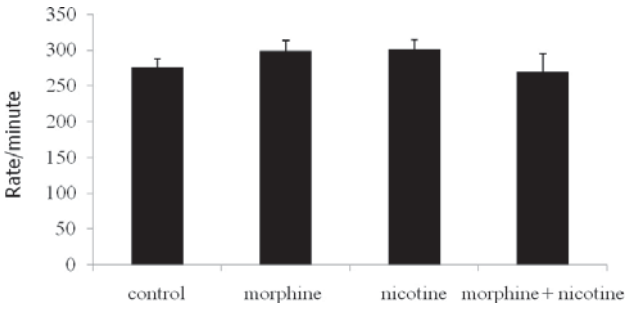


FIGURE 2. Variation in HR in experimental groups.

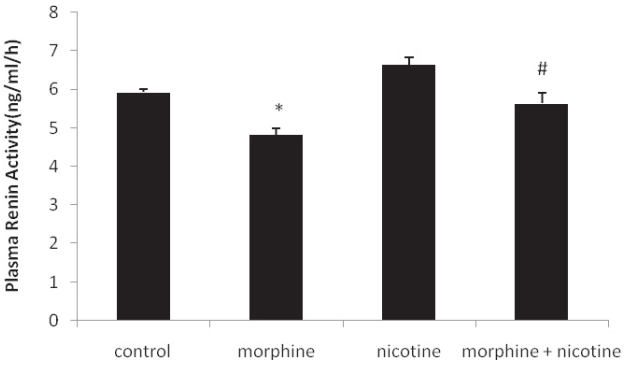


FIGURE 3. Variations of PRA in experimental groups.
*Significant difference between morphine with all other three groups ($p<0.05$).
# Significant difference between morphine+ nicotine with morphine and nicotine groups (all $p<0.05$).

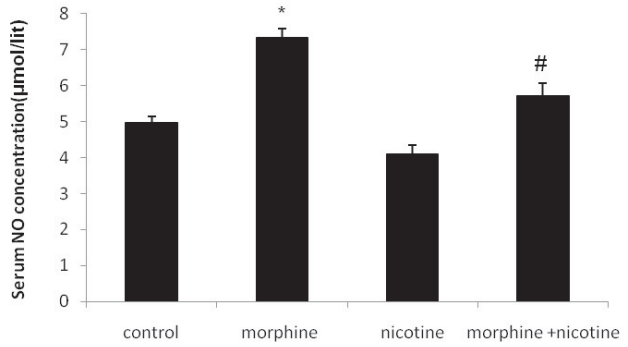


FIGURE 4. Variations in serum level of NO in experimental groups.
*Significant difference between morphine with all other three groups ($p<0.05$).
# Significant difference between morphine+ nicotine with morphine and nicotine groups ($p<0.05$).

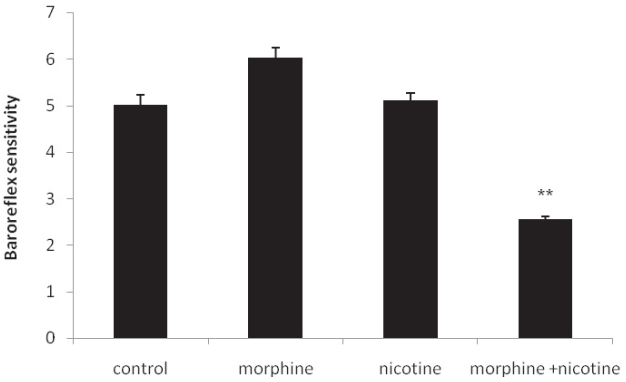


FIGURE 5. Variations in BRS in experimental groups.
**Significant difference between morphine+ nicotine with all other three groups (all $p<0.01$).

## RESULTS

*BP and HR*

The SBP, DBP and MAP were decreased significantly in morphine combined + nicotine group compared to vehicle and nicotine groups ($p<0.01$). Administration of morphine was also significantly decreased DBP, MAP and SBP compared to vehicle group. HR was not significantly differed among groups (Figure 1 and 2).

*PRA measures*

The PRA was significantly higher in morphine + nicotine group compared to morphine treated animals ($p<0.01$). PRA in morphine group was decreased compared to vehicle group ($p<0.001$) while, in nicotine group it was higher than vehicle group (Figure 3).

*Serum NO concentration*

The serum concentration of NO was increased in morphine treated animals compared to vehicle. In morphine + nicotine group the serum level of NO was also higher than vehicle group ($p<0.05$) however, in nicotine treated animals it decreased compared to vehicle group ($p<0.05$) (Figure 4).

*Effects of morphine and nicotine on BRS*

In morphine treated group the BRS was increased compared to vehicle group ($p<0.05$). Treatment with nicotine has no significant effect on BRS (compared to vehicle) while, this index was significantly decreased in morphine + nicotine group compare to all groups (all $p<0.05$) (Figure 5).

## DISCUSSION

Present findings demonstrated that prolonged co-administration of nicotine and morphine decreased PRA and blood pressure in hypertensive rats while administration of nicotine increased PRA and blood pressure in these animals. In addition, in these animals, the serum concentra-

tions of NO were increased following chronic consumption of morphine alone or in combination with nicotine. Accumulating evidences indicated that in early phase of 2K1C hypertensive animals both increased activity of PRA and renin-angiotensin-aldosterone and sympathetic activity are responsible for increasing blood pressure [14, 15, 16]. In addition, subsequent to eight weeks of clipping, altered endothelial function is also of notable factors in regulation of hypertension. Nicotine has established to enhance the Ang-II receptor mediated vasotrophic effect [17, 18], BP response to angiotensin II (Ang-II) and increase Ang II–induced contractions of aortas and mesenteric arteries [18]. Our results also demonstrated that application of nicotine increased BP and PRA in 2K1C hypertensive animals. NO is certainly one of the most important endothelium originated factors. NO is a derivative of L-arginine which is converted in L-citrulline and NO. NO has multiple beneficial functions in vascular homeostasis including vascular tone and modulation of BP. Endothelial dysfunction, which is described as decreased NO bioavailability, is considered among important risk factors for hypertension. Abnormalities in endothelium-derived relaxing factors especially NO and impaired endothelium-dependent relaxation in human clinical and several animal models of hypertension have been well demonstrated [19-21]. A recent study indicated that morphine stimulated secretion of NO and anti-hypertensive role has been reported for endogenous morphine-NO signaling events [22]. Therefore, morphine possibly modulates endothelial function and vascular endothelial cells function and influences the local paracrine-autocrine regulatory pathway by endogenously expressed authentic morphine, thus, causes constitutive NO expression. Moreover, considerable evidences revealed that the NO signalling plays paramount role in opioid receptor-mediated responses in the neurocardiovascular system [23, 24]. It has been well documented that the smoking behavior is of the main risk factors for coronary vascular diseases including, hypertension. Chronic smoking impairs endothelial function by inhibiting the formation of NO in parallel with increasing the degradation of NO via generation of free oxygen radicals [5].Furthermore, nicotine down-regulates eNOS, an enzyme involved in the pathway of NO generation, decreases endothelium dependent vasodilatation, and in turn stimulates the adhesion of leukocytes to the endothelium to induce VED and further atherosclerosis [25-27]. In addition, nicotine induces hypertension by stimulating the release of catecholamine [28]. Our results demonstrate that following concomitant administration of morphine and nicotine the serum NO concentration was more than the group that received only nicotine. So, one can conclude that morphine may inhibited the effects of nicotine on NO in hypertensive rats.

In current set of experiments we also examined the effect of co-administration of morphine and nicotine on BRS and observed a decreased in BRS in hypertensive rats. Some studies indicated that endogenous and exogenous opioids regulate the performance of BRS through effects on central opioids receptors [9]. In habitual smokers, besides vasopathic [29] and myopathic processes, alterations in the function of the autonomic blood pressure control should be considered as possible cause of cardiovascular diseases [30]. Abnormalities of the vascular barorecptors as well as glossopharyngeal (the vagal) nerve can be responsible for the loss of BRS [31]. During these pathologic circumstances, the nervous BP control can be abolished so that extreme fluctuation of ABP may occur. Indeed, recently smoking-induced alterations of the autonomic nervous system, especially a disturbance of the sympatho-vagal balance, were reported [32]. During this two suggested probable mechanisms for this sympatho-vagal imbalance: Chronic nicotine (tobacco) abuse leads to a restricted sensitivity of the afferent baroreceptors or directly restricts the baroreflex-centers in the brain stem [7]. While a diminished perfusion of the brain leading orthostatic hypotension, an elevated sympathetic tone corresponds to reduce BRS [32], the limited compensation of the smoke induced catecholamine-peaks causes a labile hypertension [31]. However, we expected morphine stimulates release of NO from endothelial cell, increase arterial compliance, hence, decreases BP and consequently improves BRS. A recent study indicated smoking increases the heart rate, systolic, mean and diastolic blood pressure, and decreases the baroreflex sensitivity [33]. Cigarette smoking increases the activity of the autonomic nervous system (the sympathetic part), increases heart rate and blood pressure and impairs the baroreflex [34, 35].The mechanism of increasing blood pressure and heart rate by nicotine is believed to be by activation of the sympathetic nervous system [36] or by effects on endothelial function [37]. Nicotine diminishes the baroreflex gain probably via reducing arterial compliance and stretch receptor responsiveness [38]. The direct interaction of nicotine with central mechanisms integrating the baroreceptor input into autonomic responses is another plausible mechanism for the depressant action of nicotine on baroreflexes [39]. A third mechanisms the ability of nicotine to modify the effectors responsiveness to reflex autonomic modulation [30]. We expect that increase arterial compliance was due to increase NO level in the combination of morphine and nicotine leading increase BRS, But we observed that BRS decrease in this group. Thus, it is possible that the decrease BRS witch was observed in the combination of morphine and nicotine was due to direct interaction of morphine and nicotine whit central mechanisms integrating the baroreflex function.

## CONCLUSION

In conclusion, this study demonstrated that prolonged combined treatment with morphine and nicotine decrease PRA and BP while increase the serum concentration of NO in hypertensive rats. Also administration of morphine prevented induction of hypertension probably via NO pathways. Co-administration of morphine and nicotine decreased BRS in 2k1c hypertensive rats probably via central nervous system.

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## DECLARATION OF INTEREST

The authors declare no conflict of interest for present study.

## REFERENCES

[1] Chelland Campbell S, Moffatt RJ, Stamford BA. Smoking and smoking cessation -- the relationship between cardiovascular disease and lipoprotein metabolism: a review. Atherosclerosis. 2008; 201(2):225-235.
[2] Lakier JB. Smoking and cardiovascular disease. Am J Med. 1992; 93(1A):8S-12S.
[3] Khalili P, Nilsson PM, Nilsson JA, Berglund G. Smoking as a modifier of the systolic blood pressure-induced risk of cardiovascular events and mortality: a population-based prospective study of middle-aged men. J Hypertens. 2002; 20(9):1759-1764.
[4] Groppelli A, Giorgi DM, Omboni S, Parati G, Mancia G. Persistent blood pressure increase induced by heavy smoking. J Hypertens. 1992; 10(5):495-499.
[5] Toda N, Toda H. Nitric oxide-mediated blood flow regulation as affected by smoking and nicotine. Eur J Pharmacol. 2010; 649(1-3):1-13.
[6] Belgore FM, Lip GY, Blann AD. Successful therapy reduces levels of vascular endothelial growth factor (VEGF) in patients with hypertension and patients with hypercholesterolemia. Atherosclerosis. 2000; 151(2): 599.
[7] Grassi G, Seravalle G, Calhoun DA, Bolla GB, Giannattasio C, Marabini M, et al. Mechanisms responsible for sympathetic activation by cigarette smoking in humans. Circulation. 1994; 90(1):248-253.
[8] Peart JN, Gross GJ. Cardioprotective effects of acute and chronic opioid treatment are mediated via different signaling pathways. Am J Physiol Heart Circ Physiol. 2006; 291(4):H1746-H1753.
[9] Thurston CL, Starnes A, Randich A. Changes in nociception, arterial blood pressure and heart rate produced by intravenous morphine in the conscious rat. Brain Res. 1993; 612(1-2):70-77.
[10] Toda N, Kishioka S, Hatano Y, Toda H. Modulation of opioid actions by nitric oxide signaling. Anesthesiology. 2009; 110(1):166-181.
[11] Waki H, Murphy D, Yao ST, Kasparov S, Paton JF. Endothelial NO synthase activity in nucleus tractus solitarii contributes to hypertension in spontaneously hypertensive rats. Hypertension. 2006; 48(4):644-650.
[12] Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide. 2001; 5(1): 62-71.
[13] Valenti VE, Ferreira C, Meneghini A, Ferreira M, Murad N, Ferreira Filho C, et al. Evaluation of baroreflex function in young spontaneously hypertensive rats. Arq Bras Cardiol. 2009; 92(3): 205-215.
[14] Martinez-Maldonado M. Pathophysiology of renovascular hypertension. Hypertension 1991; 17(5):707-719.
[15] Nystrom HC, Jia J, Johansson M, Lambert G, Bergstrom G. Neurohormonal influences on maintenance and reversal of two-kidney one-clip renal hypertension. Acta Physiol Scand. 2002; 175(3):245-251
[16] Ozaykan B, Dogan A. Effects of salt loading on sympathetic activity and blood pressure in anesthetized two-kidney, one clip hypertensive rats. Bosn J Basic Med Sci.; 11(4):228-233.
[17] Xiao D, Xu Z, Huang X, Longo LD, Yang S, Zhang L. Prenatal gender-related nicotine exposure increases blood pressure response to angiotensin II in adult offspring. Hypertension. 2008; 51(4):1239-1247.
[18] Li JM, Cui TX, Shiuchi T, Liu HW, Min LJ, Okumura M, et al. Nicotine enhances angiotensin II-induced mitogenic response in vascular smooth muscle cells and fibroblasts. Arterioscler Thromb Vasc Biol. 2004; 24(1):80-84.
[19] Klagsbrun M, D'Amore PA. Vascular endothelial growth factor and its receptors. Cytokine Growth Factor Rev. 1996; 7(3):259-270.
[20] Nicosia RF, Nicosia SV, Smith M. Vascular endothelial growth factor, platelet-derived growth factor, and insulin-like growth factor-1 promote rat aortic angiogenesis in vitro. Am J Pathol. 1994; 145(5):1023-1029.
[21] McCall TB, Boughton-Smith NK, Palmer RM, Whittle BJ, Moncada S. Synthesis of nitric oxide from L-arginine by neutrophils. Release and interaction with superoxide anion. Biochem J. 1989; 261(1):293-296.
[22] Sigmon DH, Carretero OA, Beierwaltes WH. Angiotensin dependence of endothelium-mediated renal hemodynamics. Hypertension. 1992; 20(5):643-650.
[23] Dortch-Carnes J, Russell KR. Morphine-induced reduction of intraocular pressure and pupil diameter: role of nitric oxide. Pharmacology. 2006; 77(1):17-24.
[24] Dortch-Carnes J, Randall KR. Morphine-induced nitric oxide production in isolated, iris-ciliary bodies. Exp Eye Res. 2009; 89(5):660-664.
[25] Tsai CH, Yeh HI, Tian TY, Lee YN, Lu CS, Ko YS. Down-regulating effect of nicotine on connexion 43 gap junctions in human umbilical vein endothelial cells is attenuated by statins. Eur J Cell Biol. 2004; 82(12):589-595.
[26] Fang Q, Sun H, Mayhan WG. Impairment of nitric oxide synthase-dependent dilatation of cerebral arterioles during infusion of nicotine. Am J Physiol Heart Circ Physiol. 2003; 284(2): H528-H534.
[27] Chalon S, Moreno H, Jr., Benowitz NL, Hoffman BB, Blaschke TF. Nicotine impairs endothelium-dependent dilatation in human veins in vivo. Clin Pharmacol Ther. 2000; 67(4):391-397.
[28] Czernin J, Waldherr C. Cigarette smoking and coronary blood flow. Prog Cardiovasc Dis. 2003; 45(5):395-404.
[29] Stefanadis C, Vlachopoulos C, Tsiamis E, Diamantopoulos L, Toutouzas K, Giatrakos N, et al. Unfavorable effects of passive smoking on aortic function in men. Ann Intern Med. 1998; 128(6):426-434.
[30] Niedermaier ON, Smith ML, Beightol LA, Zukowska-Grojec Z, Goldstein DS, Eckberg DL. Influence of cigarette smoking on human autonomic function. Circulation 1993; 88(2):562-571.
[31] Robertson D, Hollister AS, Biaggioni I, Netterville JL, Mosqueda-Garcia R, Robertson RM. The diagnosis and treatment of baroreflex failure. N Engl J Med. 1993; 329(20):1449-1455.
[32] Lucini D, Bertocchi F, Malliani A, Pagani M. A controlled study of the autonomic changes produced by habitual cigarette smoking in healthy subjects. Cardiovasc Res. 1996; 31(4):633-639.
[33] Gross ER, Hsu AK, Gross GJ. Opioid-induced cardioprotection occurs via glycogen synthase kinase beta inhibition during reperfusion in intact rat hearts. Circ Res. 2004; 94(7):960-966.
[34] Gerhardt U, Hans U, Hohage H. Influence of smoking on baroreceptor function: 24 h measurements. J Hypertens. 1999; 17(7):941-946.

[35] Lucini D, Bertocchi F, Malliani A, Pagani M. Autonomic effects of nicotine patch administration in habitual cigarette smokers: a double-blind, placebo-controlled study using spectral analysis of RR interval and systolic arterial pressure variabilities. J Cardiovasc Pharmacol. 1998; 31(5):714-720.

[36] Cryer PE, Haymond MW, Santiago JV, Shah SD. Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. N Engl J Med. 1976; 295(11):573-577.

[37] Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J, et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. Circulation. 1993; 88(5 Pt 1):2149-2155.

[38] Giannattasio C, Mangoni AA, Stella ML, Carugo S, Grassi G, Mancia G. Acute effects of smoking on radial artery compliance in humans. J Hypertens. 1994; 12(6):691-696.

[39] Ashworth-Preece M, Jarrott B, Lawrence AJ. Nicotinic acetylcholine receptors in the rat and primate nucleus tractus solitarius and on rat and human inferior vagal (nodose) ganglia: evidence from in vivo microdialysis and [125I]alpha-bungarotoxin autoradiography. Neuroscience. 1998; 83(4): 1113-1122.