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Intra-arterial Infusion of Leptin does not Affect Blood Pressure in Salt-loaded Rabbits

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Abstract: The aim of this research is to see the effect of intra-arterial infusion of leptin on blood pressure of salt loaded rabbits *in vivo*. Increased blood pressure was produced in rabbits by giving diets containing 8% sodium chloride for 5 weeks. Leptin in different concentrations was infused intra-arterially into rabbits fed on high salt diets and the response was compared in rabbits fed with low salt diets. High salt diets produced significant increase in blood pressure. In rabbits fed with low salt diet, leptin infused intra-arterially caused an increase in blood pressure while infusion of leptin into rabbits fed with high salt diets does not affect the blood pressure. In conclusion, salt loading to rabbits abolishes the effect of leptin on cardiovascular system. This may indicate that leptin effect on sympathetic activity is altered by high salt diets in these animals.

Key words: High salt diet, cardiovascular system, sympathetic activity, leptin, blood pressure

INTRODUCTION

Leptin is a polypeptide hormone, produced in and secreted by adipose tissue, discovered almost 15 years ago (Zhang *et al.*, 1994). Successive studies that followed its discovery demonstrated that leptin causes body loss in rodents through sympathetic activation of brown adipose tissue and oxidation of fatty acids (Collins *et al.*, 1996) but lately it has been shown that leptin has obvious effects on cardiovascular system. Cardiovascular diseases like stroke, chronic heart failure, coronary heart disease and acute myocardial infarction were associated with elevated serum leptin (Rahmoui and Haynes, 2004; Beltowski, 2006; Mohamad *et al.*, 2009). On blood vessels, leptin has two opposing effects. First one is neurogenic presser effect through activation of sympathetic nervous system causing vasoconstriction and increasing mean blood pressure and heart rate (Rahmoui and Haynes, 2004). It has been found that Leptin decreased arterial pressure after suppression of sympathetic influence using ganglionic blockade (Fruhbeck, 1999) or chemical sympathectomy (Lembo *et al.*, 2000). The second effect of leptin is direct vasodilatation that tends to decrease arterial blood pressure through stimulation of nitric oxide from endothelial cells (Kimura *et al.*, 2000) or through endothelial-independent manner (Nakagawa *et al.*, 2002). Endothelial cells have leptin receptors (Lembo *et al.*, 2000; Sierra-Honigmann *et al.*, 1998) and leptin administration in rat causes a dose-dependent increase in NO metabolite concentrations. Fruhbeck (1999) showed infusion of leptin during inhibition of NO synthesis increased arterial

pressure in anesthetized rats. *In vitro* studies have shown that leptin evokes an endothelium-dependent relaxation of mesenteric arteries and veins (Mohammed *et al.*, 2007) and aortic ring (Kimura *et al.*, 2000) and both actions are by a mechanism involving endothelial release of nitric oxide. In human, Matsuda *et al.* (2003) reported 40% increase in coronary blood flow in patients underwent cardiac catheterization after leptin infusion and this increase in blood flow is also endothelial-independent. The vasodilatation effect of leptin is abolished in anesthetized rats after administration of N^G-nitro-L-arginine-methylester (L-NAME) (Lembo *et al.*, 2000) and in isolated mesenteric arteries obtained from salt loaded rats (Jaffar *et al.*, 2005). The purpose of this study is to explore the acute effect of intra-arterial administration of leptin on blood pressure in salt loaded rabbit.

MATERIALS AND METHODS

This study was done in Department of Physiology, Jordan University of Science and Technology. The study was carried out from September 2008 to March 2010.

Experiments were performed in 60 New Zealand white rabbits of either sex, weight ranged between 1.5 and 2.5 kg. All procedures were approved by Jordan University of Science and Technology animal research committee.

Procedures: 30 rabbits were salt-loaded by feeding them high salt diet containing 8% NaCl and water given ad libitum for 5 weeks (Jaffar *et al.*, 2005). The other 30

rabbits were feed normal food contained 0.4% NaCl and water given ad libitum and this group was used as control group (low salt).

Study design: At the end of feeding period, the animals were anesthetized with 30 mg kg⁻¹ sodium pentobarbital via circumflex ear vein. This dose is supplemented as required. A polyethylene catheter was inserted into right common carotid artery for measurement of blood pressure, heart rate and for infusion of leptin. The trachea was cannulated and artificial respiration was instituted so that eucapnia was maintained through out the experiment. Blood pressure and heart rate were recorded by Harvard universal oscillograph. Mean arterial blood pressure (MABP) and Heart Rate (HR) were recorded every 10 min over 30 min after administration of the hormone. The pH was monitored to make sure that anesthetized rabbits have adequate ventilation.

The 30 high salt and low salt rabbits were divided into three group comprising 10 animals each. First group was infused with 3 microgram kg⁻¹, second with 5 microgram kg⁻¹ and the third with 7 microgram kg⁻¹.

Statistical analysis: Data are expressed as Mean±SD. Statistical differences were calculated using unpaired student's test to compare between values of blood pressure and heart rate after administration of the last leptin dose given with the basal values. The p<0.05 was considered as significant.

RESULTS AND DISCUSSION

At the end of feeding period the weight of the low and high salt rabbits were 2.2 kg (range, 1.6

to 2.65 kg, n = 30) and 2.1 kg (range, 1.65 to 2.8 kg, n = 30), respectively.

The mean blood pressure increased (from 65±4 to 90±7 mmHg, p<0.01) in rabbits fed with high salt diet. Mean blood pressure was significantly increased in low salt fed rabbits after infusion of leptin in all concentrations used (Table 1) and the heart rate did not changed significantly. In salt loaded animal infusion of leptin showed no effect on mean blood pressure and heart rate (Table 2).

High salt diets caused significant increase in blood pressure in anesthetized rabbits. This is also reported by others (Salahdeen and Alada, 2007). Present study results showed no effect of acute intra-arterial infusion of leptin at different concentrations on heart rate and mean arterial pressure in salt loaded hypertensive anesthetized rabbits. This is also demonstrated *in vitro* study by Jaffar *et al.* (2005) where salt loading to rats completely abolished the effect endothelial-mediated vasorelaxation of leptin. In our previous study (Talafih *et al.*, 2009) leptin infused intra-arterially caused an increase in mean blood pressure and this effect was attributed to activation of sympathetic system by leptin. This increment was not seen in salt loaded hypertensive rabbits. This may indicate that the vasoconstriction effect of leptin (through it's effect on sympathetic stimulation) is abolished. This is obvious since there was no increase in heart rate after leptin infusion. So the two opposing effects (i.e., pressor effect through sympathetic activation and the hypotensive effects) of leptin on vascular system were not demonstrated in salt loaded hypertensive rabbits.

Shek *et al.* (1998) have shown that chronic infusion of leptin caused an increase in both heart rate and MBP, but this effect is firstly seen after day fourth of infusion.

Table 1: The response of the mean arterial blood pressure (mmHg) and heart rate in low salt rabbits to intra-arterial infusion of leptin at different concentration (3, 5 and 7 µg kg⁻¹) compared with baseline reading (before infusion)

Results	MABP			HR		
	3 µg kg ⁻¹	5 µg kg ⁻¹	7 µg kg ⁻¹	3 µg kg ⁻¹	5 µg kg ⁻¹	7 µg kg ⁻¹
Basal reading	65±4	66±7	66±10	273±40	273±35	266±42
10 min	71±6	71±6	72±10	272±30	275±47	270±31
20 min	73±6	72±6	74±9	280±33	280±29	26±39
30 min	75±8	75±8	75±9	283±28	272±35	274±36
p-value	0.003	0.001	0.001	0.44	0.74	0.65

p-value is the difference between baseline reading and last reading

Table 2: The response of the mean arterial blood pressure (mmHg) in high salt rabbits to intra-arterial infusion of leptin at different concentration (3,5 and 7 µg kg⁻¹) compared with baseline reading (before infusion)

Results	MABP			HR		
	3 µg kg ⁻¹	5 µg kg ⁻¹	7 µg kg ⁻¹	3 µg kg ⁻¹	5 µg kg ⁻¹	7 µg kg ⁻¹
Basal reading	90±7	87±4	90±3	225±22	222±23	244±22
10 min	88±7	87±4	92±1	221±21	220±20	245±18
20 min	89±6	86±2	91±2	224±20	223±21	244±21
30 min	89±6	84±4	88±1	222±19	226±22	239±21
p-value	0.73	0.11	0.33	0.75	0.69	0.61

p-value is the difference between baseline reading and last reading

They suggested that leptin has antidiuretic effects through its action on renal sympathetic nerves activity. Renal sympathetic stimulation by leptin would be expected to raise arterial pressure by causing vasoconstriction and by increasing renal tubular sodium reabsorption. The mean arterial pressure is slowly and progressively increased by leptin due to sympathoactivation (Dunbar *et al.*, 1997). However other studies showed leptin had diuretic and natriuretic (Jackson and Li, 1997; Villarreal *et al.*, 1998). Through these effects, one can predict that leptin decreases blood pressure. In this study, the blood pressure did not reduce after leptin infusion may be due to fact that leptin was given in one shot and it is not chronically elevated in order to show its renal effect. Another possibility for no effect for leptin on blood pressure in hypertensive rabbits was that the dose we used was not high enough to show leptin effect. Mitchell *et al.* (2001) showed that acute intravenous administration of leptin did not change arterial blood pressure.

The conflicting results in literature in regard to leptin's vascular action and its effects on blood pressure hemostasis lead one to speculate that in particular circumstances, the increased sympathetic nervous activity by leptin might be balanced by direct effects of leptin on blood vessel wall resulting in no change in arterial pressure. Our results could be explained by previous findings (Rahmouni and Haynes, 2004; Kimura *et al.*, 2000; Nakagawa *et al.*, 2002) that showed that the infused leptin intra-arterially affected both centrally (sympathetic activation resulting in increased blood pressure) and peripherally (vasodilatation resulting in decreased blood pressure). Also our results may reflect the effect of anesthesia or the acute effect of leptin on vascular system.

In conclusion, salt loading to rabbits abolishes the effect of leptin on cardiovascular system. This may indicate that leptin effect on sympathetic activity is altered by high salt diets in these animals.

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