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THE MECHANISMS OF THE ANTIHYPERTENSIVE ACTION OF HEPARIN IN SPONTANEOUSLY HYPERTENSIVE RATS

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The aim of this study was to examine the effects of heparin on blood pressure in spontaneously hypertensive rats (SHR). Chronic subcutaneous administration of heparin consistenly lowers blood pressure in hypertensive rats. This antihypertensive effect is related at least in part to a concomitant decrease in hematocrit. Spontaneously hypertensive rats were treated with subcutaneous heparin (700U/day) for 6 weeks. At the end of this period we determined cardiac output and total peripheral resistance. Weekly determinations of systolic blood pressure (tail-cuff) and hematocrit were done. Peripheral plasma renin activity, plasma aldosterone, plasma prostaglandins, and urinary kallikrein were measured. Blood pressure responses of acute and chronic heparin treatment to vasoconstrictor substances, including angiotensin I, angiotensin II and norepinephrine, were determined. Heparin produced a significant decrease in hematocrit and a parallel decrease in blood pressure. A significant increase in plasma renin activity was found in heparin treated SHR, but plasma aldosterone level significantly decreased. Plasma prostaglandins and urinary kallikrein levels were not different among the groups. The blood pressure responses to vasoactive substances were similar among the heparin treated and control groups. The results suggest that a reduced aldosterone level contributes to the antihypertensive mechanism of heparin.

Key words: heparin, hypertension, renin-angiotensin-aldosterone system, prostaglandins, kallikrein

INTRODUCTION

Prolonged heparin treatment lowers blood pressure in hypertensive rats (Mandal et al., 1978; Wilson et al., 1981; Sušić et. al., 1988), but the mechanism of its antihypertensive effect has not been fully explained. Heparin may reduce blood pressure through one or more mechanisms: (1) In rats chronically treated with heparin, hematocrit decreases in parallel with the reduction in blood pressure (Purkerson et al., 1967; Sušić et al., 1982;Olson, 1984). This decrease in

hematocrit either by affecting blood viscosity, or by affecting oxygen supply to the tissues with consequent vasodilatation, lowers total peripheral resistance, and hence blood presure. (2) It is possible that heparin exerts its effect through action on the renin-angiotensin-aldosterone system, as it is known to inhibit renin-angiotensinogen reaction (Sealey et al., 1967) and aldosterone secretion (Abbott et al., 1986). Heparin also induces lipolysis (Mayes, 1981), and thereby makes available more prostaglandin precursors; thus heparin's antihypertensive action may be mediated by increased prostaglandin production. The present study was initiated to examine the possible role of the renin angiotensinal-dosterone system and prostaglandin synthesis in mediating the antihypertensive role of heparin. To this end, the effect of chronic heparin treatment on blood pressure, hematocrit, cardiac output, plasma renin activity (PRA), plasma aldosterone, plasma PGF_{2a}, PGI_2, thromboxane (TXA_2), and urinary kallikrein were examined in SHR. In addition, in a separate group of animals the effect of heparin on blood pressure response to vasoactive substances was studied.

MATERIALS AND METHODS

Experiments were performed in adult male SHRs. All animals were given standard laboratory chow (Veterinarski Zavod, Zemun, Yugoslavia) and tap water ad libitum. Rats were divided into two groups.

Group I: Control, untreated SHRs (C-SHR) (19 rats)

Grour II: Heparin-treated (700 U/d/rats subcutaneously) SHRs (H-SHR) (17 rats)

In all rats, initial determinations of systolic blood pressure (tail-cuff), heart rate, hematocrit, and body weight were done three times in a span of 2 weeks and respective treatments commenced threafter. Blood pressure, heart rate, hematocrit, and body weights were measured once a week during the 6-week during the 6-week course of the experiment. During the fifth week of the study,six rats from each group were placed in metabolic cages and left for 2 days to adapt. Afterward, 24-hour urine samples were collected for kallikrein determination.

At the end of the 6-week period, all rats were guillotined and blood was collected (during the first 4 seconds after decapitation) into prechilled centrifuge tubes containing Na_2 ethylenediaminetetra-acetic acid (to reach a final concentration of approximately 1 mg/mL) for determination of PRA and plasma aldosterone concentration; and Na_2 ethylenediaminetetra-acetic acid and indomethacin (to reach final concentrations of about 1 mg/mL and 0.1 mg/mL respectively) for determination of PGF_{2a} and PGI_2, and TXA_2-. The samples were centrifuged at 0^0C for 15 minutes and the plasma separated and stored at -70^0C until assayed. Plasma renin activity, plasma aldosterone level, and PGI_2 were determined in 12 randomly selected plasma samples from each group, whereas the concentration of PGF_{2a} and of TXA_2 were measured in samples in which enough plasma had remained.

Urinary kallikrein activity was mesured by a colorimetric procedure. The method employs the synthetic substrate, Val-Leu-Arg-pNA.2HC1 (S-2266, AB

Kabi Diagnostica, Uppsala, Sweden). Incubation was carried out at 37^{0}C and pH 8.2. Plasma renin activity and plasma aldosterone concentration were determined by radioimmunoassay, using the Phadebas Angiotensin 1 test (Pharmacia AB, Uppsala, Sweden) and Aldoctk-125-M (Sorin, Biomedica, Saluggia, Italy), respectively. Before the prostaglandin assay, a solid-phase extraction procedure was employed, using SEP-PAK C_{18} columns (Waters Associates, Milford, MA). For estimation of 6-keto-PGF_{1a} and TXB_2 (nonenzymatic breakdown products of PGI_2 and TXA_2, respectively) radioimmunoassay kits from Amersham (Buckinghamshire, England) (RPA 515 and RPA 516) were used, whereas for determination of PGF_{2a}, a kit from Pharmatrade (Budapest, Hungary) was employed. All determinations of kallikrein, PRA, aldosterone, prostaglandins I_2 and F_{2a}, and TXA_2 were run in duplicate.

In the second experiment, the effect of heparin on cardiac output (CO) (Coleman's modified method, Coleman, 1974), total peripheral resistance (TPR) and blood pressure response to vasoactive agents was examined. Three groups rats were studied, with six to eight rats in each group:

1. Control, untreated SHRs

2. SHRs treated with a single intravenous injection of heparin (200 units), 30 minutes before study

3. SHRs treated with heparin for 5 weeks(700 U/d/rat subcutaneously)

Rats were anesthetized with pentobarbital (35mg/kg, i.p.), and a tracheal cannula (PE-50, Clay Adams, Parsippany NY) using a low-volume displacement transducer (P23Db, Statham, Oxnard, CA) and a direct recorder (Physiograph Four, Narco Bio Systems, Inc., Houston, TX). For injection of drugs, a catheter was placed in the jugular vein. Angiotensin I (AI, 5ng), angiotensin II (AII, 5ng) and norepinephrine (Nor, 50ng) were injected through a venous catheter in a volume of 0.1mL and the blood pressure recorded. Each drug was injected twice and the average value calculated.

Statistical analyses were done according to Steel and Torie (1960). Bonferroni's modifications of Student's test (Wallenstein at al., 1980) for multigroup comparison was used to test significance of differences among the groups. The results were expressed as means ± ISEM.

RESULTS

There was no difference in body weight among the groups at the beginning or at the end of the study. In figure 1 is shown a significant ($P<0.01$) reduction in systolic blood pressure, with a parallel decrease in hematocrit in heparin-treated SHRs during the 6 weeks of the experiment.

In SHRs with chronically decreased hematocrit a significant fall in mean arterial pressure (MAP) was observed. Cardiac output was found to be increased and total peripheral resistance to be decreased in heparin treated rats as compared to controls (Table I).

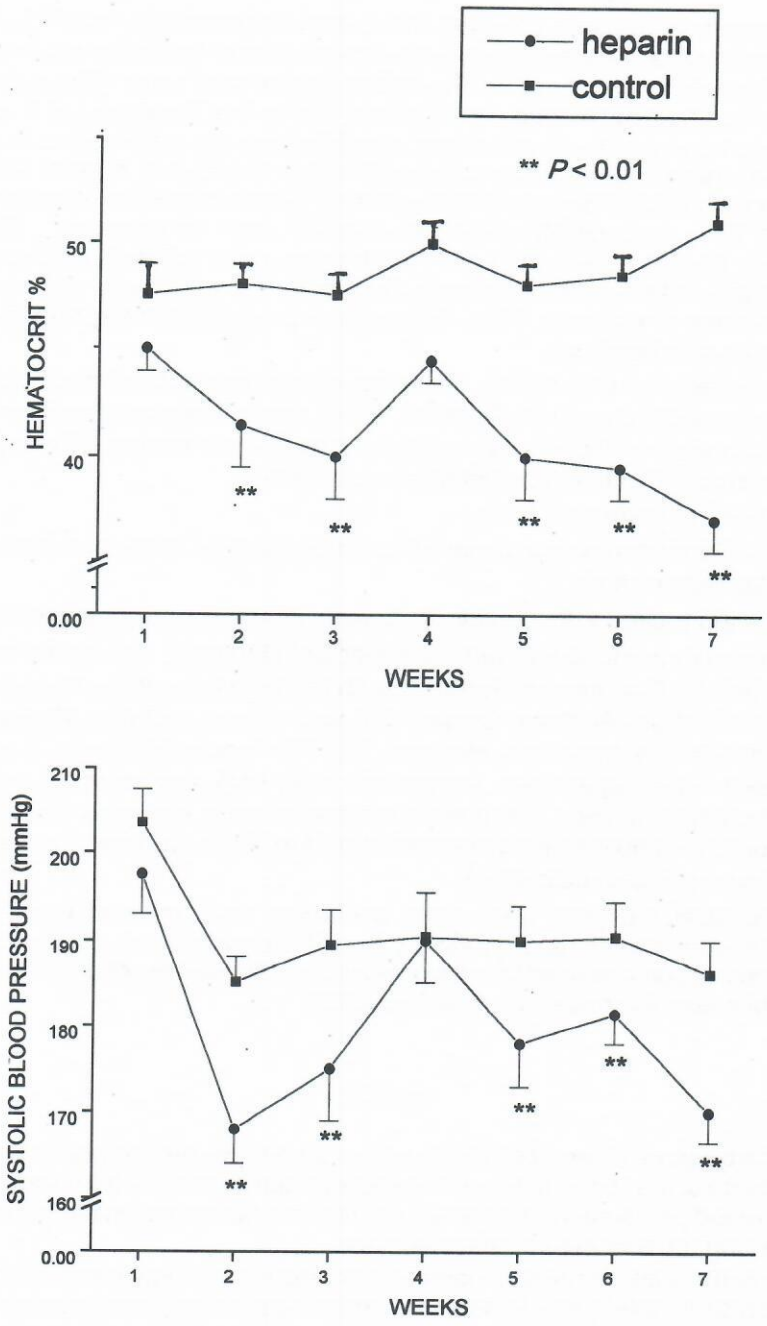

Figure 1. Systolic blood pressure and hematocrit in control and heparin treated SHR during 6 weeks of the experiment

Table 1. Hemodynamic parameters in SHR after decrease of the hematocrit

Groups	Hct (%)	MAP (mmHg)	CO (ml/min/100g)	TPR (mmHg/ml/min/100g)
Control (n=8)	48 ± 1	201 ± 3	23 ± 2	7.8 ± 0.4
Heparin (n=8)	38 ± 2*	175 ± 4*	35 ± 3*	4.6 ± 0.3*

n = number of animals in group
*$P < 0.05$

Plasma renin activity showed a significant ($P < 0.001$) increase in heparin treated rats as compared with control animals and heparin induced a significant ($P < 0.01$) decrease in plasma aldosterone (Figure 2).

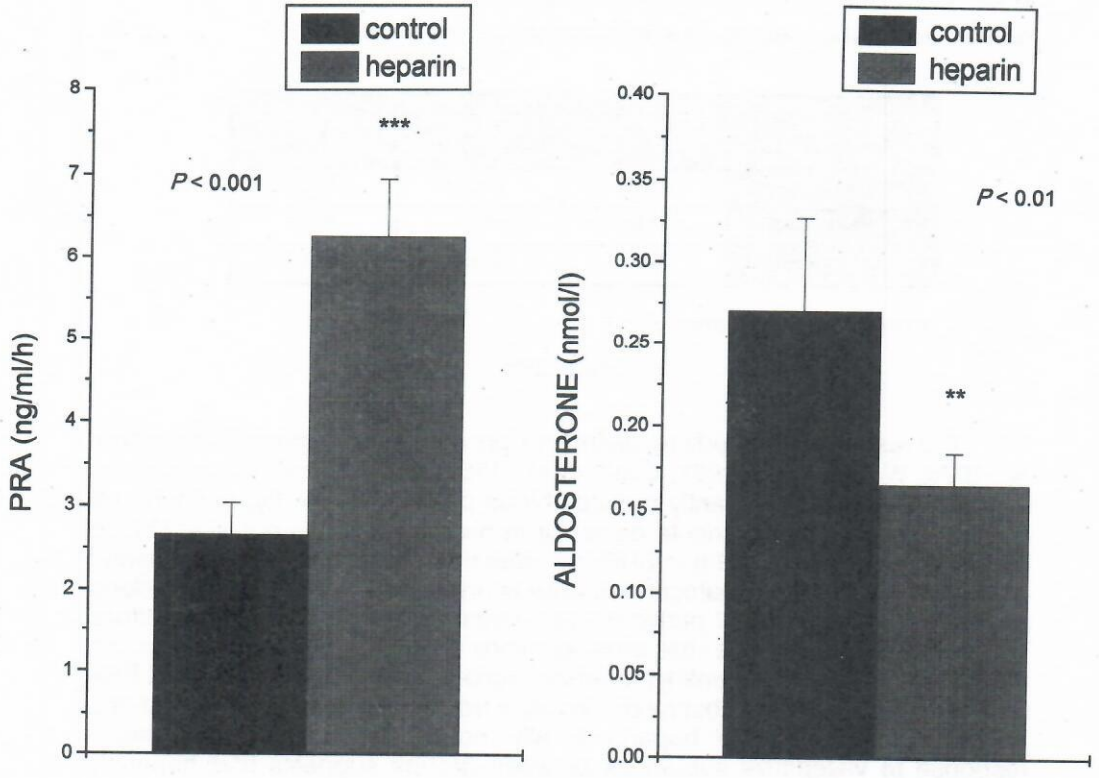

Figure 2. Plasma renin activity and plasma aldosterone concentration in SHR at the end of treatment

Table II shows plasma concentrations of PGF_{2a}, PGI_2 and TAX_2, and urinary kallikrein excretion in control and heparin treated rats. Heparin produced only a significant ($P < 0.05$) increase in plasma TXA_2 level and did not affect any of the others variables measured.

Table 2. Plasma concentrations of prostaglandins and urinary kallikrein excretion in SHR.

Groups	$PGF_{2\alpha}$ (pg/ml)	PGI_2 (pg/ml)	TXA_2 (pg/ml)	kallikrein (U/24hr)
Control	664 ± 65 (7)	35 ± 3 (12)	225 ± 4 (11)	1.8 ± 0.2 (6)
Heparin	638 ± 12 (5)	36 ± 4 (10)	474 ± 87* (9)	2.0 ± 0.1 (6)

*$P < 0.05$
Number of animals for each determination is given in parentheses

Blood pressure responses to intravenous injections of angiotensin I, angiotensin II and norepinephrine in control and heparin treated rats are presented in Table III and were similar between the groups.

Table 3. Blood pressure response to a single dose of AI, AII and Nor in SHR

Groups	Blood pressure response (mmHg)		
	AI	AII	Nor
Control (n=7)	31 ± 2	33 ± 2	27 ± 2
Single heparin (n=6)	34 ± 4	40 ± 5	30 ± 3
Chronic heparin (n=8)	32 ± 2	28 ± 2	25 ± 2

n= number of animals in group

DISCUSSION

The results of this study reconfirm the previous observations (Purkersonet al., 1976; Wilson et al., 1981; Sušić et al., 1984), that chronic subcutaneous heparin treatment significantly reduces blood pressure in the hypertensive rat models. The fact that an acute decrease in hematocrit does not affect blood pressure (Sušić et al., 1988) in SHR indicates that the blood pressure lowering effect of the chronic hematocrit decrease is not due to a direct effect of blood viscosity changes on total peripheral resistance and implies an indirect effect. The present data indicate that prostaglandins or kallikrein kinin systems are unlikely mediators of the antihypertensive action of heparin. The elevated PRA and plasma TXA_2 levels observed in heparin treated rats may even abolish the antihypertensive effect of heparin. Finally, no difference in blood pressure response to vasoactive subtances between groups suggests that heparin's antihypertensive effect is not manipulated by these vasoactive subtances.

It has been reported that, in rats with malignant renal hypertension, chronic heparin treatment reduces PRA (Kamitsuji et al., 1986; Olson, 1984) and that heparin inhibits kallikrein activity (Nasjletti and Malik, 1984). These findings appear to be at variance with our results. However, the results in this study only demonstrate that heparin does not affect urinary kallikrein excretion, but do not rule out an effect of heparin on plasma kallikrein. The increase in PRA observed in heparin treated SHR in our study may be related to a simultaneous decrease in blood pressure. Apparently, reduction in blood pressure within the renal hemodynamic autoregulatory range induces renin relase through a prostaglandin-dependent mechanism (Freeman et al., 1984).

At the end, heparin treatment resulted in a significant reduction of plasma aldosterone level despite similar elevation of PRA. This finding is consistent with the previous observations (Abbott et al., 1986), and suggests that the antihypertensive effect of heparin is due to suppression of aldosterone. Heparin has been shown to inhibit angiotensin II-induced aldosterone secretion in the rat (Azukizawa et al., 1988) and may also explain the antihypertensive effect of heparin.

Since lowering of hematocrit influences renal hemodynamics, filtration and postglomerular oncotic pressure (Schrier and Anderson, 1980) it may be suggested that all these factors shift the renal function curve to the left; i. e. the same sodium and water excretion would occur at a lower blood pressure level. The result of such a change would be lowering of blood pressure, as found in the present study.

There is abundant proof that exogenous heparin has a potent vasodepressor effect, and implies a role for endogenous heparin in physiological control of blood pressure. Results of recent studies indicate that heparin inhibited the proliferation of vascular smooth muscle cells from SHR and Wistar-Kyoto rats (Sato et al., 1995) which contribute to the development of hypertension or vascular hypertrophy in SHR. It has been shown that heparin binds to human vascular endothelial cells and could inhibit endothelin-1 production (Letourneur et al., 1995). Thus, further investigations are essential to elucidate the antihypertensive mechanism of heparin, and to determine whether heparin can predictably lower blood pressure in hypertensive patients, as in the hypertensive rats.

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MEHANIZMI ANTIHIPERTENZIVNOG DEJSTVA HEPARINA KOD PACOVA SA SPONTANOM HIPERTENZIJOM

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SADRŽAJ

Cilj ovog rada bio je ispitati efekte heparina na krvni pritisak kod pacova sa spontanom hipertenzijom (SH). Dokazano je da hronično s. c. aplikovanje

heparina snižava krvni pritisak kod pacova sa hipertenzijom. Ovaj antihipertenzivni efekat je delom povezan sa sniženjem hematokrita. SH pacovi bili su tretirani heparinom (700U/dan, s. c.) u toku 6 nedelja. Posle ovog perioda određivan je minutni volumen i ukupni periferni otpor. Sistolni krvni pritisak (indirektna metoda) i hematokrit kontrolisani su nedeljno. Nivo renina, aldosterona i prostaglandina određivani su u plazmi, a kalikreina u urinu. Merena je promena krvnog pritiska nakon davanja vazoaktivnih supstanci, angiotenzina I i II i norepinefrina, kod akutno i hronično heparinom tretiranih pacova. Heparin dovodi do značajnog pada hematokrita i krvnog pritiska. Kod SH pacova tretiranih heparinom utvrđen je značajan porast reninske aktivnosti u plazmi, za razliku od aldosterona čija vrednost značajno pada. Između grupa nema razlike u nivou prostaglandina u plazmi i kalikreina u urinu. Krvni pritisak meren posle davanja vazoaktivnih supstanci nije se bitno razlikovao kod pacova tretiranih heparinom i pacova u kontrolnoj grupi. Ovi rezultati ukazuju da svoje antihipertenzivno dejstvo heparin ostvaruje snižavanjem nivoa aldosterona u plazmi.