EFFECT OF SINGLE INTRAVENOUS DOSE OF FUROSEMIDE ON CARDIAC AUTONOMIC ACTIVITY IN RABBITS

By

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ABSTRACT

Cardiac functions are controlled by higher brain centers and sympathetic and parasympathetic nervous systems, which act via adrenergic and muscarinic receptors respectively, Cardiac baroreceptor, renal baroreceptors and arterial baroreceptors influence reflexly the activity of cardiovascular autonomic nerves. The potent loop diuretic furosemide is used for rapid relief of acute pulmonary edema due to acute left sided heart failure and congestive heart failure. So the present work was conducted to evaluate the effect of single intravenous dose of furosemide on cardiac autonomic activity in rabbits.

Seventy-two rabbits were randomly divided into three equal major groups with different autonomic st-

ates; one with intact autonomic components, the second with blocked parasympathetic components (0.2 mg/kg I.V. atropine), the third with blocked sympathetic (1 mg/kg I.V. atenolol). Each major group include four subgroups; control (non furosemide treated with intact kidneys; furosemide treated with intact kidneys; non furosemide treated binephrectomized) and furosemide treated binep-hrectomized. Binephrectomized grou-ps allow the assessment of furosemide autonomic activities independent of furosemide renal effects. Our data demonstrate modulation of cardiac baroreceptor reflexes activity by furosemide that is independent of furosemide renal effects. However, furosemide had no effect on isolated rabbit heart. Also, we found that furosemide induced an activation of tonic sympa-

thetic activity, which could be explained by either renin angiotensin system activation or central effect of furosemide on sympathetic outflow. This observation could be of great value in treatment of acute left sided heart failure where alteration in automatic nervous system function has documented. In addition, we have observed an enhancement of the baroreceptor reflex sensitivity of the mid part of the sigmoid curve in rabbits treated with beta adrenergic blocker; atenolol which could point to a probable advantage for combining furosemide with beta blocker.

INTRODUCTION

Loop diuretics blocks the $Na^+ K^+$ 2Cl- co transport in the thick ascending limb of the loop of Henle and therefore, induce volume depletion by increasing the renal excretion of solutes and water[1]. However, there is evidence that the effect of furosemide in acute left sided heart failure, is not only due to volume depletion but there is probable extrarenal action of furosemide because amelioration starts rapidly before development of diuresis[2]. It produces short-term hemodynamic effects independent of natiuresis[2,3,4,5]. It was reported that furosemide had stimulant effect

on corticosterone release in binephrectomized rats; this was suggested to be centrally mediated through the pituitary hypothalamic axis[6]. Furosemide affects brains GABAergic neurotransmission; it shares GABA its action on $Na^+ K^+$ 2Cl- cotransport mechanism on neuronal cell membrane[7]. Consequently, central mechanism of furosemide could be extended to the central mechanism of stress induced autonomic and neuroendocrine responses. It remains unclear how the probable change in cardiac autonomic activity may occur during treatment with furosemide. This probable alteration could be of great importance in the management of critically ill cardiac patients as in acute left sided heart failure or myocardial infarction where alteration in autonomic nervous system function has been documented[8]. So, we tried to assess the autonomic activity after single intravenous dose of furosemide in rabbits. The assessment was conducted through quantification of the heart rate response to acute pharmacologically induced blood pressure variations using phenylepherine as vasopressor agent and sodium nitroprusside as vasodepressor agent[9]. Estimation of bradycardic response to blood pressure increase will reflect

reflex parasympathetic activity, while estimation of tachycardic response to blood pressure decrease will reflect reflex sympathetic activity.

MATERIALS AND METHODS

Drugs :

1) Furosemide; Lasix ampoules 40 mg/ml (Hoechset).
2) L-phenylepherine powder (Sigma).
3) Sodium nitroprusside powder vial; 50 mg, (David Bull Laboratories).
4) Atropine sulfate powder, (MacFarlan Smith LTD-Edinburgh).
5) Atenolol, powder (Sigma).
6) Thiopental-sodium powder, 500 mg (E.P.I.CO).

Methods :

The studies were performed on New Zealand White rabbits of either gender, housed in metal cage under similar housing conditions and weighting 1-1.5 kg. Rabbits were allowed free access to food and water.

Assessment of furosemide cardiac autonomic activities :

In this study, assessment of the cardiac autonomic activity was realized through measurement of the heart rate (HR) response to the pharmacologically induced blood pressure variations. These variations were induced by the vasopressor phenylepherine and the vasodepressor sodium nitroprusside. Phenylepherine[10] was injected slowly at different doses (10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80 µg/0.1ml). Sodium nitroprusside[10] was also injected slowly at different doses (1,2,3,4,5,6,7,8,9,10 µg/0.1ml) .

Animal grouping

Seventy-two rabbits were randomly divided into three equal major groups with different autonomic states; one with intact autonomic components, the second with blocked parasympathetic, the third with blocked sympathetic. Each major group include four subgroups; control non furosemide treated of intact kidney; furosemide treated of intact kidney; non furosemide treated binephrectomized and furosemide treated binephrectomized. Binephrectomized groups allow the assessment of furosemide autonomic activities independent of furosemide renal effects.

Binephrectomy was done according to the method of Lang[14]. Full atropinization was verified by absent reflex bradycardia with the phenylephrine induced blood pressure

increase of 10-20 mmHg[13]. Complete cardiac B1 receptor block was verified by absent reflex tachycardic response to sodium nitroprusside induced decrease in blood pressures of 10-20 mmHg[10,13]. Because the HR response to atropine or atenolol was maximal and stable after 15 minutes, this time was selected as the standard time for HR measurement [12,13].

Group I : rabbits with intact autonomic status
Group Ia: control (non furosemide treated + intact kidneys)
Group Ib: furosemide treated (2mg/kg)[11] + intact kidneys
Group Ic: non furosemide treated + binephrectomized
Group Id: furosemide treated + binephrectomized
Group II: rabbits with blocked parasympathetic (0.2 mg/kg atropine I.V)[12]
Group IIa: control (non furosemide treated + intact kidneys)
Group IIb: furosemide treated + intact kidneys
Group IIc: non furosemide treated + binephrectomized

Group IId: furosemide treated + binephrectomized
Group III: rabbits with blocked sympathetic component (atenolol 1mg/kg I.V)[13]
Group IIIa: control (non furosemide treated + intact kidneys)
Group IIIb: furosemide treated + intact kidneys
Group IIIc: non furosemide treated + binephrectomized
Group IIId: furosemide treated + binephrectomized

Analysis of the mean arterial pressures (MAP)-HR baroreflex relationship
The resting and vasoactive induced response values for HR and MAP were fitted by non-linear regression analysis to a sigmoidal logistic equation (9,15). Fig (1) represents a sigmoid plot of the cardiac baroreceptor reflex response variables. Transformation of each value of MAP and HR variation through the mean basal value was performed for unifying all the basal values for all the induced variations.

A sigmoidal logistic equation with four parameters had been proposed[15,16] where p1 is the lower

(bradycardia) plateau, p_2 is the HR range between the plateaus, $p_1 + p_2$ is the upper (tachycardia) plateau, p_3 is a curvature coefficient that is a range-independent measure of gain, and p4 is the median blood pressures (BP50, mm Hg) at the point half way between plateaus. The average gain (G) was given by G= P_2 x P_3 x 0.22. The average curve of a group of rabbits was reconstructed from means of the different curve parameters.

Assessment of cardiac baroreceptor reflex autonomic response :

For each rabbit, two marginal ear veins were cannulated for drug injection and the central ear artery was cannulated for measurement of mean BP (diastole +1/3 pulse pressure) and HR. The arterial cannula was connected to pressure transducer coupled to an amplifier its signals were monitored through a computer system (intracept TSD 286,England). Glass syringes containing phenylepherine hydrochloride and sodium nitroprusside were connected to the two marginal ear veins cannulae for injections. Both basal MAP and HR and the peak level of blood pressure and HR variations obtained by the vasoactive drugs were recorded. With every

dose, both MAP and HR were allowed to return to basal values before the next dose of the vasoactive drug was injected. Stimulus-response curves relating mean arterial pressure (MAP) to HR were constructed according to the method of Korner et al.,[9] as modified by Head and McCarty[16] using about 24 different levels of blood pressure variations. Basal mean arterial pressure was elevated in a stepwise fashion from 75 mm HG to 160 mm Hg by I.V. injection of phenylephrine in different doses and then was lowered by I.V. injection of sodium nitroprusside in different doses. At least 30 minutes elapsed between infusion of each vasoactive to allow MA Pand HR to return to the baseline values.

Assessment of cardiac basal tonic autonomic activities :

This was realized through measurement of the basal heart rate shift under conditions of blocked sympathetic innervations using atenolol[13] and blocked parasympathetic innervations using atropine[12]. The bradycardia with atenolol indicates the degree of sympathetic tone and the tachycardia with atropine indicates the degree of parasympathetic tone in the different groups.

Effect of furosemide on isolated rabbit heart :

This was done according to the modified Langendorff's method[17]. The hearts were quickly suspended and perfused at 37°c via the aortic root with Ringer–Locke solution. In all studies, the Ringer Lock solution was delivered from a reservoir at a constant rate and temperature of 37°c. The normal myocardial contraction was recorded and the furosemide was injected in the cannula in the following doses: 10, 100, 1000, 2000, 4000, 8000 µg[18], each dose was applied twice. After each application, the preparation was allcwed to recover for 15 minutes.

Statistical analysis :

For analysis of baroreflex ANOVA was done followed by post hoc Tukey test according to Rifenburg [19].

Unpaired-t-test was done for the difference between P2 (heart rate range) of control group and P2 of the corresponding sum of atropine + atenolol. Paired-t-test was done for the difference between P4 (mid point of sigmoid curve) and MAP values for each group.

Area under the curve (AUC) was estimated for each group under the

three different conditions of autonomic states P<0.05 was considered significant. All statistical analysis were performed with SPSS for window 10.

RESULTS

Haemodynamic resting values and parameters of cardiac baroreflex curves :

Mean basal arterial pressure and HR values obtained before any treatment were 75.8±3 mmHg and 292.6±21.5 beat/min respectively.

Cardiac baroreflexes were sigmoidal with an average upper plateau of 334.7±14.5 beat/min, lower plateau of 190.3±14.3 beat/min, range of 144.3±3.0 beat/min and average gain of 1.6± 0.121 beat/min/mmHg.

Effect of furosemide on cardiac tonic autonomic activity and basal MAP and HR :

In rabbits with intact ANS (table1-a), furosemide treatment had induced non-significant modification of mean basal blood pressure, in non-nephrectomized and nephrectomized rabbits (gp Ib,Id) but induced an increase in basal HR in nephrectomized rabbits (gp Id).

Fully atropinized groups of rabbits (Table 1-b) showed significant de-

crease in mean basal blood pressure in nephrectomized rabbits either furosemide treated or non-treated compared to non nephrectomized (gpIIC,IId vs IIa, IIb), all fully-atropinized groups of rabbits had significant increase in basal blood pressure compared to corresponding groups with intact autonomic activity except the non-treated nephrecto-mized (gpIIc vs Ic). While the basal HR of fully-atropinized groups showed non-significant difference when compared with those of corresponding control groups of intact ANS except a significant increase of basal HR in non-nephrectomized furosemide treated rabbits (gp IIb vs Ib).

Under complete block of cardiac β_1 adrenergic receptor with atenolol table (1-c); nephrectomy induced significant decrease in mean basal blood pressure in either furosemide treated or not furosemide treated compared to non-nephrectomized groups (gp IIIc. IIId vs IIIa, IIIb).

In table 1-c when atenolol treated groups were compared to corresponding group with intact ANS or blocked parasympathetic component, it was found that there was significant decrease in basal blood pressure in

group IIId. Groups with complete cardiac B1 receptor block showed nearly equal basal heart levels when compared to each other, while they were significantly lower when compared with the corresponding groups of either fully atropinized or those with intact ANS.

Effect of furosemide on reflex cardiac autonomic activity :

In rabbits groups with intact autonomic components, the lower bradycardia plateau (P1) associating the vasopressor effect of phenylepherine was significantly decreased only in furosemide treated nephrectomized rabbits (Table1-a, Fig.2). While the upper tachycardia plateau associating sodium nitroprusside injection were nearly equal (Table 1-a, Fig.2). Also, when comparing the amplitude of the sigmoid (P2), a significantly smaller amplitude levels were noted with furosemide treated groups nephrecto-mized or non-nephrectomized (gp Ib,Id). This decrease in amplitude translate the upward shift of the lower plateau (Fig.2).

In fully atropinized groups of rabbits, there was non-significant change in value of P2 except the significant decrease in non-treated nephrecto-

mized group when compared with treated non-nephrectomized (Table 1-b). While significant decrease in P2 were noted with the different groups of full atropinization when compared with those of corresponding groups with intact ANS except in case of treated nephrectomized (gp IId vs Id).

When examining the value of P2 in groups with complete block of β_1 adrenergic receptors, it was found that nephrectomy induced a significant decrease compared to the non furosemide-treated non-nephrectomized group, while all atenolol treated group had significant decrease in P2 when compared to the corresponding groups of intact ANS.

When comparing P2 of the baroreceptor reflex response under condition with intact autonomic component to the sum of P2 of the vagal and sympathetic components (Fig.4), it was found that furosemide treated groups either nephrectomized or non nephrectomized showed significant increase in sum of both P2 atropine plus P2 atenolol compared to P2 of corresponding groups with intact ANS. This increase was significant only when furosemide treatment was combined with nephrectomy. While

non-treated groups either nephrectomized or non-nephrectomized showed significant decrease in sum of both P2 atropine plus P2 atenolol as compared to P2 of corresponding group with intact ANS (Fig.4).

For P3 value which quantifies the steepness of cardiac response of baroreflex activity, table (1-a) showed non significant change among the different groups with intact ANS. While the full atropinized groups of rabbits showed significant increase of P3 in both nephrectomized groups either furosemide treated or non treated (gp IId vs Id).On other hand, in rabbits with complete B1 adrenergic, it was found that furosemide treated groups either nephrectomized or non-nephrectomized showed significant increase of P3 values   (table 1-c).When comparing P3 in full atropinized rabbits and P3 of rabbits with blockade of β adrenergic receptors to the corresponding P3 under conditions of intact ANS, there was significant increase (Table 1-a, b, c).

Examining P4, which represents the midpoint of the sigmoid at which the gain is maximum, it was noticed that the MAP of P4 significantly lower with nephrectomized-non-treated rab-

bits (Table 1-a) and nephrectomized treated or non-treated in both full atropinized and atenolol treated groups (table1-b,1-c).

Comparing P4 level under either atenolol or atropine treatment to that of intact ANS (Table 1-b,c), group IIb showed only significant upward shift of P4. In group IIId, there was downward shift of P4 level in comparison to that of intact ANS (gpId) or atropinized rabbits (gpIId). While significant decrease was detectable with furosemide treated non-nephrectomized rabbits (gpIIIb) under atenolol, when compared with corresponding full atropinized group (gp IIb).

The average gain of baroreceptor reflex activity showed a significant decrease of its value only in group Id. Interestingly group IId had a significant increase of average gain (Table1-a,b) .

Fig.3 a,b,c,d illustrated the percentages of AUC in the three different conditions of autonomic activities. These percentages point to the approximate midway of the curve of intact ANS to that of separate vagal and sympathetic activities in the non-treated non-nephrectomized group (Fig.3-a).This relation seems to be preserved with furosemide treatment of non nephrectomized (Fig.3-b).On the other hand,the nephrectomized groups (Fig.3 c,d) show coaptance of the sigmoid of intact ANS activity with that representing the activity of the separate sympathetic component.There was no evident changes on isolated rabbit's heart when perfused with Ringer lock's solution containing different concentration of furosemide.

Table 1.(a) Effect Of Single I.V. dose Of Furosemide (2mg/kg) On The Sigmoid Curve Parameters Indicative Of Autonomic Activity Of The Different Groups (m±sem,n=6)

groups	P1 bpm	P2 bpm	P3	P4 mmHg	G bpm/mmHg	Gmax bpm/mmHg	BHR bpm	MBP mmHg	P1+P2 bpm
Control (1a)	190.3±14.3	144.3±3.0	0.051±0.004	90.5±3.2	1.6±0.121	1.84±0.15	292.6±21.5	75.8±3.0	334.7±14.5
Furosemide (1b)	242.7±12.9	99.6±7.7[*]	0.055±0.012	86.4±1.6	1.2±0.023	1.3±0.12	315.4±7.3	78.3±3.0	342.3±10.8
Neph (1c)	253.9±25.1	119.8±8.4	0.058±0.014	80.2±2.6[*]	1.5±0.120	1.7±0.11	331.5±5.6	70.7±3.6	373.8±28.3
Furosemide +neph (1d)	289.5±16.2[*]	70.2±4.9[*&]	0.062±0.008	83.2±1.6	0.95±0.014[&]	1.1±0.11[*]	338.6±14.5[*]	70.9±1.0	359.7±19.0

(table1.a) intact autonomic activity

Table 1.(b) Effect Of Single I.V. dose Of Furosemide (2mg/kg) On The Sigmoid Curve Parameters Indicative Of Autonomic Activity Of The Different Groups (m±sem,n=6)

groups	P1 bpm	P2 bpm	P3	P4 mmHg	G bpm/mmHg	Gmax bpm/mmHg	BHR bpm	MBP mmHg	P1+P2 bpm
Control (IIa)	285.3±26.3$^{(v)}$	62.1±6.0$^{(v)}$	0.16±0.028$^{(v)}$	92.98±1.9	2.24±0.34$^{(v)}$	2.5± 0.27$^{(v)}$	343.1±23.7	88.2±3.7$^{(v)}$	347.3±24.0
Furosemide (IIb)	324.7±13.5$^{(v)}$	67.6±6.4$^{(v)}$	0.13±0.012$^{(v)}$	92.4±.9$^{(v)}$	1.9±0.19l	2.2±0.21	380.6±12.6$^{(v)}$	86.9±1.6$^{(v)}$	392.3±14.4$^{(v}$
Neph (II C)	300.2±17.6$^{(v)}$	46.4±2.7$^{(v#)}$	0.27±0.027$^{(v*#)}$	82.5±1.4$^{(v#)}$	2.8±0.540$^{(v)}$	3.1±0.22$^{(v)}$	332.2±18.1	73.6±1.4$^{(v#)}$	346.6±19.6
Furosemide +neph (IId)	314.6±9.9	60.5±4.8	0.28±0.029$^{(v*#)}$	80.8±1.1$^{(v#)}$	3.7±0.497$^{(v*#)}$	4.1±0.37$^{(v*#o)}$	366.4±6.7	75.2±1.1$^{(v*#o)}$	375.02±10.0

(table1.b) blocked parasympathetic (atropine)

Table 1.(c) Effect Of Single I.V. dose Of Furosemide (2mg/kg) On The Sigmoid Curve Parameters Indicative Of Autonomic Activity Of The Different Groups (m±sem,n=6)

(table 1. c) β_1 adrenergic blockade (atenolol)

groups	P1 bpm	P2 bpm	P3	P4 mmHg	G bpm/mmHg	Gmax bpm/mmHg	BHR bpm	MBP mmHg	P1+P2 bpm
Control (IIIa)	171.3±12.5[a]	58.2±3.8[ψ]	0.14±0.008[ψ]	90.3±0.86	1.8±0.196	2.1±0.15	220.1±13.1[ψ]	77.1±1.5	232.8±12.2[ψω]
Furosemide (IIIb)	176.4±7.2[ψω]	50.6±4.4[ψ]	0.195±0.024[*ψω]	82.2±1.1[C*ω]	2.2±0.17[ψ]	2.5±0.18	215.6±4.3[ψω]	79.7±.3	227.1±5.0[ψω]
Neph (IIIc)	188.8±7.0[a]	39.02±2.1[ψ]	0.16±0.010[ψω]	76±1.8[*ω]	1.44±0.073[θω]	1.6±0.14[θω]	217±7.4[ψω]	64.8±1.7[*#ψω]	227.8±6.8[ψω]
Furosemide +neph (IIId)	178±4.8[ψω]	49.5±3.7[ψ]	0.19±0.013[C*#ψω]	70.2±1.1[C*#ψω]	2.1±0.260[ψω]	2.4±0.21[ψω]	219±4.6[ψω]	66.9±.6[*#ψω]	227.5±4.4[ψω]

P1: lower plateau, P2: heart rate range, P3: curvature coeffecient, P4: midpoint of the sigmoid curve, BHR:basal heart rate, MBP: mean basal blood pressure, G: average gain, Gmax: maximal gain, P1+P2: upper plateau, bpm: beat per minute .

*: $p < 0.05$ versus control (non treated non nephrectomized group within the same autonomic state), # $p < 0.05$ versus treated non nephrectomized group within the same autonomic state, $: $p < 0.05$ versus control non treated nephrectomized group within the same nephrectomized group within the same autonomic state, Ψ: $p < 0.05$ versus corresponding gp of intact autonomic state, ω: $p < 0.05$ versus corresponding group of blocked autonomic state,Ψ: $p < 0.05$ versus corresponding group of blocked parasympathetic

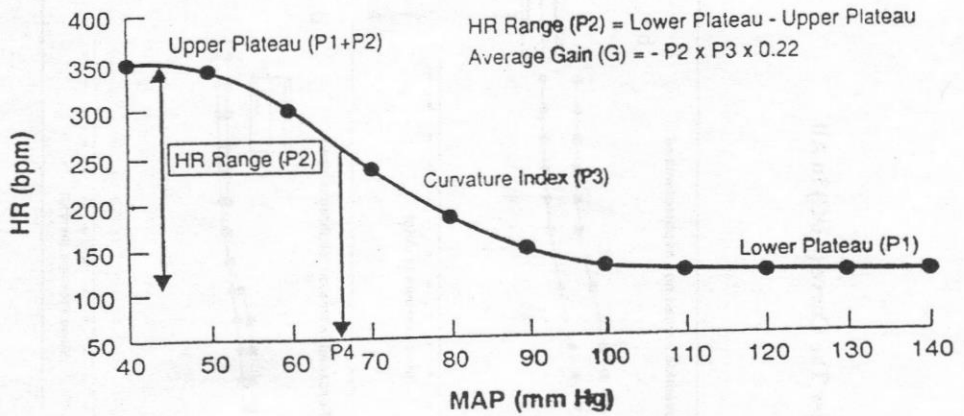Double way ANOVA followed by post hoc Tuckey test

Fig.1. Parameters of sigmoid curve of baroreceptor reflex HR response

Fig.2. Effect Of Single I.V. dose Of Furosemide (2mg/kg) on Sigmoid Curves Of
Baroreceptor Reflex Autonomic Activity In Groups With Intact Autonomic Component
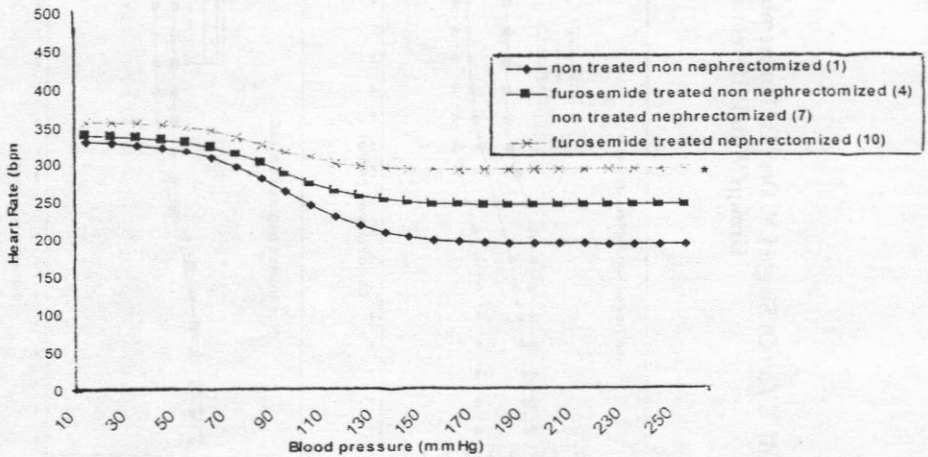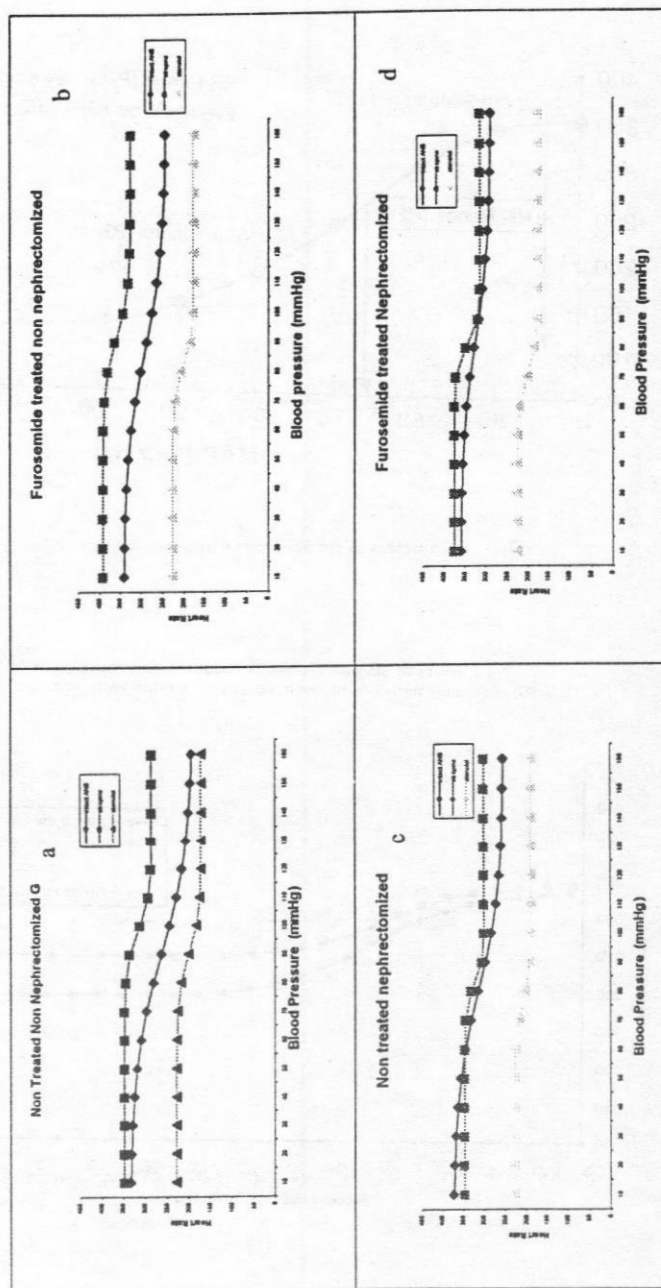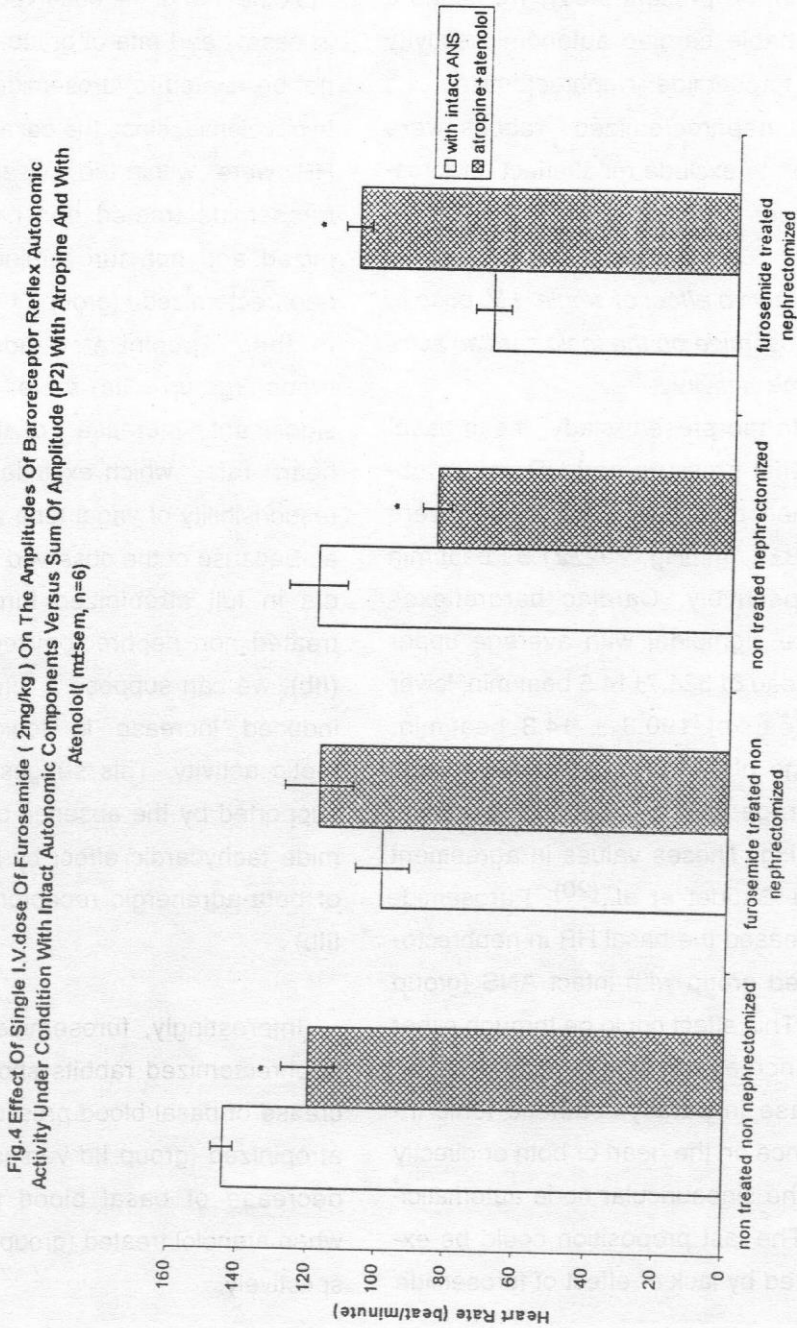
Fig. 3. (a,b,c,d) Effect Of Single I.V. Dose Of Furosemide (2mg/kg) On Area Under The Curve (AUC) In All Groups With Different Autonomic Conditions

Fig.4 Effect Of Single I.V.dose Of Furosemide (2mg/kg) On The Amplitudes Of Baroreceptor Reflex Autonomic Activity Under Condition With Intact Autonomic Components Versus Sum Of Amplitude (P2) With Atropine And With Atenolol(m±sem, n=6)

DISCUSSION

In the present study; we tested a probable cardiac autonomic activity for furosemide. Nephrectomized and non nephrectomized rabbits were used to exclude renal effect of furosemide.

In vivo effect of single I.V. dose of furosemide on the tonic cardiac autonomic activity :

In the present study, mean basal arterial pressure and HR values obtained before any treatment were 75.8±3 mmHg, 292±21.5 beat/min respectively. Cardiac baroreflexes were sigmoidal with average upper plateau of 334.7±14.5 beat/min, lower plateau of 190.3 ± 14.3 beat/min, range of 144.3 ± 3.0 beat/min and average gain of 1.6±0.121 beat/min/mmHg. Theses values in agreement with Gaudet et al.,[20]. Furosemide increased the basal HR in nephrectomized group with intact ANS (group Id). This effect could be through either an increase in sympathetic or a decrease in parasympathetic tonic influence on the heart or both or directly on the sinoauricular node automaticity. The last proposition could be excluded by lack of effect of furosemide on the isolated rabbit heart. On the other hand, the observed increase in basal heart rate of group (Id) could not be related to furosemide induced hypovolemia, since the basal levels of HR were within the basal value of furosemide treated non nephrectomized and non furosemide treated nephrectomized (groups I-b and c). In the atropinizated non-treated group (group IIa) there was non significant increase of the basal heart rate, which exclude also the responsibility of vagal tone withdrawal. Because of the observed tachycardia in full atropinized furosemide-treated non nephrectomized rabbits (IIb), we can suppose a furosemide induced increase in tonic sympathetic activity. This suggestion was supported by the absence of furosemide tachycardic effect by blockade of beta-adrenergic receptors (group IIIb).

Interestingly, furosemide treated nephrectomized rabbits show an increase of basal blood pressure when atropinized (group IId versus Id) and decrease of basal blood pressure when atenolol treated (group IIId) respectively.

The present suggestion of an increased tonic sympathetic activity by furosemide is in agreement with the study of Francis et al.,[21]. They reported that there was an activation of sympathetic nervous system and an increase of plasma arginine vasopressin, after intravenous furosemide treatment in congestive heart failure[22]. The mechanism of increased sympathetic nervous system activity seen in patients with congestive heart failure treated by furosemide is unkown but had been attributed at least in part to facilitation by angiotensin II[21].

Other investigators reported that furosemide acutely increased plasma renin activity[22]. The stimulation of renin angiotensin system by furosemide is suggested to be mediated by a direct intrarenal effect on the macula densa, activation of baroreceptor mechanism through diuresis-induced volume contraction, and through renal PG system which modulate the rate of renin secretion[23,24]. Peripheral presynaptic Ang II receptor had been suggested to mediate this sympathetic activation by the renin angiotensin system.[25].

The route of administration seemed to be important, I.V. treatment consistently raised plasma renin activity at 10 to 20 minutes while oral treatment had no effect in patients with heart failure despite the preserved stimulus of volume contraction and sodium excretion promoted by diuresis[21].

Effect of furosemide on the amplitude of the reflex autonomic activity :

In our study, furosemide decreased the amplitude (P_2) of reflex HR response (Table 1-a). This decrease in the amplitude appears to result mainly from attenuation of the reflex bradycardic response (P_1) and it was majorated in nephrectomized rabbits. Because P1 represents mainly the reflex vagal activity, this attenuation could be explained by a decrease in reflex vagal activity but P_1 value of the curve representing the separate vagal component did not differ (gp IIIb, IIId, gp IIIa). The need for a coexistance of two active vagal and sympathetic can explain this alteration of reflex vagal activity by furosemide. This hypothesis can be supported when comparing the amplitude of the

reflex HR response in condition of intact ANS with the sum of the separate sympathetic and vagal amplitudes (Fig.4). It indicates the lesser amplitude in case of intact ANS than the sum of the amplitudes of the separate autonomic components by furosemide which become significantly manifesting with nephrectomy. This inhibitory action of furosemide on the cardiac vagal reflex activity has not been previously discussed,however there are some bibliographic data which can support the presence of reflex vagal inhibitory action induced by furosemide. Furosemide has been shown to inhibit the bronchoconstrictor response to exercise[26], inhaled allergen[27], distilled water[28], sodium metabisulphate[29], and adenosine[30]. These bronchocostrictor responses were suggested to be partially mediated by vagus. Furosemide produced a concentration-dependent inhibition of the electrical field stimulation-induced cholinergic contraction. This inhibition was suggested to be mediated through a prejunctional mechanism[31]. The proposed mechanism on airway nerves was inhibition of the $Na^+K^+2Cl^-$ cotransporter or release of endogenous cyclooxyge-

nase products [31]. Although the physiological function of cotransport in nerves is not known, it may regulate ion gradient that determine the resting membrane potential. In the previous study of Verleden et al.,[31] the inhibitory action of neurally induced contraction by furosemide was still present when the air way epithelium was removed. The observation supported more neuronal target for furosemide through $Na^+ K^+ 2Cl^-$ cotransport. It is therefore possible that inhibition of this cotransport may affect the resting membrane potential, making the threshold voltage for depolarization more difficult to reach.

Gamma amino butyric acid (GABA) shares furosemide its action on Cl^- channels[32]. It have been shown to inhibit the cholinergic and noncholinergic neurotransmission in guinea pig air way suggesting a presynaptic modulation of the release of neurotransmitter[33]. It has been demonstrated that GABA decreases the contractile response of airway smooth muscle to cholinergic nerve stimulation by inhibiting the evoked release of acetylcholine[34].

Effect of furosemide on the steepness (slope) of reflex heart rate response to induced blood pressure variation :

The steepness of heart rate response to blood pressure variation (P_3) quantify the cardiac response to minimal B.P variation. The greater the response, the greater will be the value of P_3. Comparing the values of P_3 of non-treated non-nephrectomized in the three groups of rabbits under the three different conditions of ANS activities,we noticed an increase of its value when the sympathetic and parasympathetic components were working alone (Table 1). This observation indicates the importance of the physiological coexistance of both autonomic components in attenuating the magnitude of cardiac response to blood pressure variations.This seems to be a physiological protective mechanism.

Neither nephrectomy nor furosemide had a significant effect on this steepness of cardiac response under condition of intact autonomic activity,but they had induced a majoration of this steepness when examining the cardiac response of the sympathetic component and parasympathetic separately. This can be of a probable importance when furosemide is given to patients with heart failure and attenuated baroreceptor reflex activity[35], where the neurohumoral abnormalities account for depressed baroreflex sensitivity.

Also, myocardial infarction (MI) patients can get benefit by this potentially effect of furosemide on baroreceptor sensitivity. Reduction in baroreflex sensitivity has been reported in few patients with MI[36], where depressed baroreflex reflects reduced vagal activity that is probably combined with elevated sympathetic activity.

Effect of furosemide on the total sensitivity of reflex heart rate response (average gain)-Role of the kidney, and CNS :

Average gain is a collective estimate of the P2 and P3. It indicates the total power of the cardiac baroreceptor reflex mechanism. This indicator appears to be decreased by furosemide in nephrectomized with intact autonomic components (Table 1-a). This decrease could indicate the physiological participation of the kid-

neys in keeping the normal gain value by furosemide, which could be through action of furosemide on renal afferent activity with consequent remodulation of the sympathetic and parasympathetic activities. On the other hand, the observed increase in atropinized groups of rabbit could be attributed largely to the previously discussed enhancement of P3 (cardiac response steepness).

Nephrectomy alone had no effect on the gain of baroreceptor reflex activity of group Ic with intact ANS. However, furosemide treatment in group Id induced a significant reduction in gain when compaed to group Ib. This can indicate the importance of the kidneys in the mediation of a normal baroreceptor reflex sensitivity with furosemide. Previous studies performed on rats also demonstrated that single I.V injection of furosemide induced increase in efferenl renal sympathetic nerve activity that was abolished in rats with bilateral nephrectomy[37]. Also, suggestion of renal participation in furosemide action, can be supported by our observation of enhanced gain of reflex sympathetic activity in nephrectomized rabbits (group II c, IId) which was illustrated more in furosemide treated (group IId). At the same time in calculating areas under the curves for the tested groups of rabbits,we observed that the curve (Fig.3 c&d) of nephrectomized group with intact ANS had taken the positional level of nephrectomized group with blocked vagal component. This could be explained by an activated reflex sympathetic mechanism more than vagal withdrawal.

Ueno et al[40] demonstrated that furosemide produce a central sympathetic stimulating effect, where they found that intravertebral artery infusion of clonidine suppressed the furosemide induced increases in plasma noradrenaline and HR. On the other hand Colombari et al. [41] found that furosemide impairs pressor responses mediated by sympathetic activation and angiotensin II. If the sympathetic nervous system is in fact involved in the responses to furosemide traetment, it also still remains unclear as to what is (are) the signal (s) to the CNS.

In vitro effect of furosemide on isolated mammalian rabbit heart :

The present study reveals that furosemide has no effect on rabbit isolated heart. This finding is in agreement with Stamfer et al.,[38] Also, it is consistent with Kelso et al.,[39] who reported that furosemide had no effect on ventricular cardiomyocytes isolated from rabbit myocardium because furosemide had no effect on peak Ca^{2+} current amplitudes. Contradictary findings have been found where Feldman[18], et al.attributed furosemide induced negative inotropic effect on isolated rabbit heart to prostaglandin mediated coronary vasoconstriction. This contradictory findings can be explained by difference in methodology because for accurate measuremetes of intropic effect of any drug on isolated perfused heart-coronary flow and HR must be hept constant.

In conclusion, single i.v. dose of furosemide had tendency to induce an increase in cardiac tonic sympathetic activity. This increase could be responsible for an attenuation of the antihypertensive effect of furosemide . This tonic sympathoexcitation is observed with enhanced reflex sympathetic activity in the midpart of the sigmoid curve (P3). Furosemide intrarenal mechanism has been suggested and central modulation of this renal mechanism has also been disscused.

On the other hand, furosemide produced a marked diminution of the reflex cardiovagal activity. So, both components of cardiac autonomic activity had been altered by a single i.v.dose of furosemide. This observation could be of great importance for a critically ill cardiac patients.There is a reported increase in mortality of patients with MI in propertion with an observed imbalance of sympathetic-parasympathetic activities[42,43].

Interestingly, an enhancement of the baroreceptor reflex sensitivity of the mid point of the sigmoid curve in rabbits group treated by atenolol points to a probable advantage for combining furosemide with beta-blocker. This combination had been advised for selected patients with CHF .

Finally, further studies are recommended for reevaluation of furose-

mide in critical ill patients. Also, more detailed experiments are needed for exploration of probable central autonomic mechanisms of furosemide.

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تأثير جرعة وحيدة من الفيوروسيمايد عن طريق الوريد على نشاط الجهاز العصبى اللاإرادى فى قلب الأرانب

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يسيطر الجهاز العصبى السمبثاوى والباراسيمبثاوى على وظائف القلب عن طريق مستقبلات الأدرينالين و المسكارين. كما يسيطر على وظائف القلب مراكز عليا فى الجهاز العصبى المركزى وهناك مستقبلات طرفية تسيطر على النشاط اللاإرادى للقلب.

يستخدم الفيوروسيمايد بكثرة فى إرتشاح الرئة الناتج عن الفشل للجهة اليسرى من القلب والأحتشاء المزمن للقلب ويقوم الفيوروسيمايد بأغلاق متلازمة الصوديوم البوتاسيوم الكلوريد فى الطرف الصاعد لأنبوبة هنل ومن ثم يساعد على تقليل حجم الدم بفقدان الماء وملح الصوديوم ومع ذلك فإن التغيرات الدموية توضح أن تأثير الفيوروسيمايد فى الإحتشاء الحاد للجانب الأيسر من القلب لاينجم فقط عن تقليل حجم الدم بل هناك أسباب أخرى خارج الكلى يمكن أن تتسبب فى ذلك. حيث أن التحسن الرائع فى إرتشاح الرئة الحاد يبدأ سريعاً قبل حدوث أدرار البول وهناك تغيرات مصاحبة فى الأفعال المنعكسة للجهاز العصبى اللاإرادى فى مرضى القلب المزمن ومن ثم فقد افترضنا دوراً للفيوروسيمايد على الجهاز العصبى اللاإرادى. لذلك فقد تم دراسة تأثير جرعة وحيدة من الفيوروسيمايد على نشاط الجهاز العصبى اللاإرادى فى القلب وقمنا بتعين هذا النشاط. وتم تعيين هذا النشاط عن طريق العلاقة بين نبض القلب والتغييرات الحادة المحدثة فى ضغط الدم بواسطة مواد باسطة كالصوديوم نيتروبروسايد ومواد قابضة كالفنيل افرين.

وقد تمت هذه الدراسة على أرانب بيضاء من نوع نيوزيلاند لتعين التأثير المحتمل على نشاط الجهاز العصبى اللاإرادى لجرعة وحيدة تعطى عن طريق الوريد من الفيوروسيمايد على القلب. وقد تم تقسيم إثنان وسبعون أرنباً الى ثلاث مجموعات رئيسية متساوية تعد كل واحدة منها حالة من حالات الجهاز العصبى اللاإرادى فالأولى يكون فيها الجهاز العصبى اللاإرادى سليماً، والثانية يتم فيها أغلاق الجهاز الباراسيمبثاوى بالأتروبين (٢ر. مجم/كجم)، والثالثة يتم إغلاق الجهاز العصبى السمبثاوى بمقفلات مستقبلات بيتـا (أتينولول ١ مجم/كجم) وقد قسمت كل مجموعة رئيسية إلى أربعة مجموعات

متساوية : أستؤصلت الكليتان فى مجموعتان ولم تستأصل فى المجموعتين الأخريين وعولجت مجموعة من كل منها بالفيوروسيمايد ٢ مجم/كجم وقد وجدنا فى هذه الدراسة أن الفيوروسيمايد ينشط الجهاز العصبى السمبثاوى ويمكن تفسير ذلك عن طريق تنشيط نظام رنين أنجيوتنسين أو عن طريق تأثير للفيوروسيمايد على نشاط الجهاز العصبى السمبثاوى المركزى، وهذه الملاحظة هامة فى معالجة الاحتشاء الحاد للجانب الأيسر للقلب الذى يضطرب فيه الجهاز العصبى اللاارادى وبالإضافة إلى ذلك فإنه قد يكون مسئولاً عن فشل الفيوروسيمايد على المدى الطويل كعلاج لضغط الدم . كما وجدنا أن الفيوروسيمايد يقلل الفعل المنعكس للعصب الحائر وهذه الملاحظة هامة لمرضى القلب حيث أنه قد وجد زيادة فى معدل الوفيات فى حالات الفشل الحاد للجهة اليسرى من القلب تتناسب مع معدل التغير فى نشاط الجهاز العصبى السمبثاوى والباراسيمبثاوى.