Relationship between arterial baroreflex, cardiopulmonary vagal reflex and renal natriuretic response to saline in conscious rabbits

Nadine MAYBAUM, Elena GORODETSKY and Marta WEINSTOCK
Department of Pharmacology, Hebrew University, Hadassah Medical Centre, Ein Kerem, Jerusalem 91120, Israel

ABSTRACT

1. We have previously shown that normotensive rabbits with a genetic impairment in arterial baroreflex sensitivity showed a delayed sodium excretion and failed to increase their renal blood flow in response to a saline infusion that did not alter blood pressure. These renal haemodynamic and excretory abnormalities were abolished by renal denervation. The present study determined the sensitivity of the cardiopulmonary baroreceptors and the renal response to a mild saline infusion in normotensive rabbits varying widely in their arterial baroreflex sensitivity.

2. Sensitivity of cardiopulmonary baroreceptors was assessed from the slope of the relationship of the change in both blood pressure and heart rate and the dose of phenylbiguanide, a stimulator of vagal afferents.

3. The change in renal blood flow and lithium and sodium excretion was measured in response to saline, infused at a rate of 0.11 ml·min⁻¹·kg⁻¹ into the ear vein. Urine was collected via a urethral catheter and renal blood flow was measured by para-aminohippurate clearance.

4. A significant correlation was found between the magnitude of the gain of the cardiac arterial baroreflex and the sensitivity of the cardiopulmonary baroreceptor response to phenylbiguanide. The latter was significantly correlated to renal blood flow and lithium clearance 60–90 min after the start of the saline infusion.

5. It was also found that in some normotensive rabbits there was a blunting of cardiovascular regulation as indicated by a reduced sensitivity of cardiopulmonary and arterial baroreceptors. This may explain their abnormal haemodynamic and natriuretic response to salt.

INTRODUCTION

In previous studies we have shown that normotensive rabbits with a genetic impairment in arterial baroreflex sensitivity developed hypertension on a 3-fold increase in dietary salt intake. This was associated with sodium retention and a failure to suppress plasma renin activity [1]. In those rabbits the rate of sodium excretion was also delayed in response to an acute saline infusion, because their renal blood flow did not increase nor was sodium reabsorption suppressed in the proximal tubule [2]. Bilateral renal denervation abolished the renal haemodynamic and excretory abnormalities [3] and prevented sodium retention and the development of hypertension by chronic salt [4]. It is possible that the renal abnormalities resulted from inadequate modulation of renal sympathetic activity via baroreceptor reflexes. In most previous studies, in which their renal blood flow did not increase nor was sodium reabsorption suppressed in the proximal tubule [2]. Bilateral renal denervation abolished the renal haemodynamic and excretory abnormalities [3] and prevented sodium retention and the development of hypertension by chronic salt [4]. It is possible that the renal abnormalities resulted from inadequate modulation of renal sympathetic activity via baroreceptor reflexes. In most previous studies, in which

Key words: arterial baroreceptors, cardiopulmonary receptors, phenylbiguanide, rabbits, renal blood flow, sodium excretion.

Abbreviations: BP, blood pressure; HR, heart rate; MAP, mean arterial pressure; PAH, p-aminohippurate; PBG, phenylbiguanide; SHR, spontaneously hypertensive rats.

Correspondence: Professor Marta Weinstock.
saline was infused to induce reflexly mediated changes in sympathetic nerve activity, mean arterial pressure (MAP) increased [5–7]. However, the rate and amount of saline infused in our rabbits were much smaller and did not alter MAP [2,3]. This makes it unlikely that arterial baroreceptors were activated in the rabbits. On the other hand, the abnormalities in the renal response to saline could have been due to reduction in the sensitivity of their cardiopulmonary receptors. These respond to minor changes in blood volume which are insufficient to alter MAP and are activated by relatively larger fluctuations in arterial blood pressure (BP) than are required to stimulate arterial baroreceptors. Stimulation of cardiopulmonary baroreceptors decreases renal sympathetic nerve activity [8,9] and increases renal blood flow and sodium excretion. It was not feasible to measure directly the contribution of cardiopulmonary receptors to the disparate responses to saline in rabbits with high and low arterial baroreceptor sensitivity by denervating the latter, since this has been shown to enhance considerably the input from the cardiac baroreceptors [10].

In addition to mechanical stimulation, cardiopulmonary receptors can be activated by phenylbiguanide (PBG), a 5-hydroxytryptamine type 3 agonist [11,12] that also induces reflex reductions in BP, heart rate (HR) and renal sympathetic nerve activity. The effects of mechanical and chemical stimulations of cardiopulmonary baroreceptors can be abolished by vagotomy. The advantage of using PBG to stimulate vagal afferents over mechanical means is that it does not activate arterial baroreceptors [9].

The aim of the present study was to see whether rabbits with impaired cardiac baroreflex sensitivity also have lower sensitivity of cardiopulmonary baroreceptors, and whether the latter can explain their abnormalities in renal blood flow and sodium excretion. This was accomplished by determining whether there was a significant correlation between the cardiovascular response to PBG and the magnitude of the increase in renal blood flow and sodium excretion in response to volume expansion by saline. The experiments were performed in normotensive rabbits varying widely in their arterial baroreceptor sensitivity. To our knowledge, this is the first time that the sensitivity of the arterial and cardiopulmonary baroreceptor reflexes, elicited both by chemical and mechanical stimulation, has been assessed in the same conscious, normotensive animals.

MATERIALS AND METHODS

Measurement of arterial and cardiopulmonary baroreflex sensitivity

Experiments were performed in 14 male and 11 female rabbits of a mixed Israeli coloured strain, aged 4–6 months, weighing 2.5–3.2 kg, according to the guidelines of the Institutional Care Committee. The animals were housed in individual cages at a room temperature of 21 ± 1 °C. Catheters were placed under local anaesthesia with 2% lignocaine in the central ear artery (for measurement of BP) and marginal ear vein (for injection of drugs). The rabbits were allowed 30 min to recover from these procedures. The maximum changes in MAP and HR were measured in response to intravenous phenylephrine and nitroglycerine, which were administered in random order of at least 6 doses per drug ranging from 1 to 15 μg/kg. Time was allowed for MAP and HR to return to their preinjection levels between each infusion (about 5–7 min). Sigmoid barocurves relating HR to MAP were constructed for each animal, from the responses to the vasoactive agents as previously described [13], using a computer program especially developed to fit the logistic function: $HR = P_l - P_u/[1 + e^{fl(MAP - \bar{P})}]$ [14]. The average gain (G) of the reflex, which represents its sensitivity [15], was estimated. This equals $-P_u \times P_l/4.56$, where $P_l$ is the upper plateau (UPL, tachycardia), $P_u$ the HR range between the plateaus, $P_l - P_u$ the lower plateau (LPL, bradycardia), $P_o$ a curvature coefficient that is a range-independent measure of the gain, and $\bar{P}$ the median blood pressure. The relationship between MAP and HR and the gains of one rabbit with low sensitivity and one with high sensitivity are shown in Figure 1. The measurements of cardiac baroreflex sensitivity were repeated 10–14 days later and the average of the two readings calculated for each rabbit.

At the end of the second measurement of cardiac baroreflex sensitivity, the rabbits were allowed to rest for 30–45 min before assessment of their cardiopulmonary baroreceptor sensitivity to PBG. MAP and HR were measured before and immediately after the intravenous injection of PBG, 5, 10, 15, 20, 30 and 40 μg/kg, in ascending order. The lower doses of PBG (5–15 μg/kg) were injected at approximately 5-min intervals, while

![Figure 1](image-url)
in order to ensure a continuous urine flow. The infusion was about twice that normally imbibed in the same time rabbits in their home cages. The amount of fluid given

\[ \text{hippurate (PAH, 83 mg)} \]

and glucose (75 mg), lithium chloride (250 mg), for measuring effective renal plasma flow. This was followed by a continuous intravenous infusion at the rate of 0.33 ml/min containing glucose (30 mg/ml), lithium chloride (2.5 \( \mu \)Eq/ml) and PAH (5 mg/ml). Sodium chloride was given at a concentration of 10–12 \( \mu \)Eq/ml (including the sodium in the hippurate) to approximate the normal oral intake of these rabbits in their home cages. The amount of fluid given was about twice that normally imbibed in the same time in order to ensure a continuous urine flow. The infusion was continued for 90 min until effective renal plasma flow and sodium excretion reached stable values after the PAH bolus. The sodium chloride content was then increased to 26–36 \( \mu \)Eq/ml, the glucose reduced to 10 mg/ml and the infusion continued for 2 h. Measurements were made every 30 min of urine volume, plasma and urinary concentration of sodium and lithium ions (by flame photometry), creatinine (for measurement of glomerular filtration rate) [16] and PAH by standard spectrophotometric procedures [17] during the control period and after the saline infusion had begun. MAP and HR were monitored continuously.

**Statistical analyses**

Correlation coefficients were calculated from regression analyses performed between the values obtained for each rabbit of cardiac baroreflex sensitivity (G) and each of the two measures of cardiopulmonary reflex sensitivity. They were also calculated between each of the above three measures and those of sodium, lithium and PAH clearance during the saline infusion. The statistical significance of these correlations was determined by means of SPSS statistical package using Spearman’s rank method.

**RESULTS**

**Relationship between arterial (cardiac) and cardiopulmonary baroreceptor sensitivities**

The resting MAP of the rabbits in this study ranged from 89–95 mmHg and the HR from 230–254 beats/min. The gain of the cardiac arterial baroreflex ranged from 2.7 to 9 beats \( \cdot \) min\(^{-1} \cdot \) mmHg\(^{-1} \). The estimations of MAP, HR and cardiac baroreflex sensitivity were reproducible as shown by the small differences between the first and second measurement in the 25 rabbits (mean difference (± S.E.M.) MAP: 1.79 ± 1.32, \( p > 0.1 \); HR: 3.0 ± 6.9, \( p > 0.6 \); baroreflex gain: 0.34 ± 0.46, \( p > 0.3 \)). A significant correlation (Spearman’s \( \rho = 0.47, p < 0.01 \), one-tailed) was found between the slopes of the change in HR and that in BP, as a function of the dose of PBG (sensitivity of cardiopulmonary reflex) (Figure 3a). There was also a significant correlation between arterial baroreflex sensitivity and that of the cardiopulmonary reflex, as determined by the decrease in BP (\( \rho = 0.524, p < 0.01 \)) (Figure 3b), or HR (\( \rho = 0.425, p < 0.05 \)), in response to PBG (Figure 3c).

**Relationship between sensitivity of cardiopulmonary receptors to chemical and mechanical stimulation**

The average values obtained for MAP, HR, glomerular filtration rate, urine volume and sodium, lithium and PAH clearances during the control period are shown in

![Figure 2 Decrease in MAP as a function of the dose of PBG in a rabbit with high sensitivity and one with low cardiac baroreflex sensitivity (BRS) as shown in Figure 1.](image)

**Measurement of the natriuretic and haemodynamic response to a saline infusion**

Two weeks after the foregoing measurements, the male rabbits were prepared with cannulae in the ear artery and vein for BP measurements and saline infusion, and in the bladder through the urethra for urine collections, as previously described [2]. After allowing the animals to recover from these procedures for 60 min, they were given an intravenous bolus injection (5 ml) containing glucose (75 mg), lithium chloride (250 \( \mu \)Eq) and p-aminohippurate (PAH, 83.5 mg), for measuring effective renal plasma flow. This was followed by a continuous intravenous infusion at the rate of 0.33 ml/min containing glucose (30 mg/ml), lithium chloride (2.5 \( \mu \)Eq/ml) and PAH (5 mg/ml). Sodium chloride was given at a concentration of 10–12 \( \mu \)Eq/ml (including the sodium in the hippurate) to approximate the normal oral intake of these rabbits in their home cages. The amount of fluid given was about twice that normally imbibed in the same time in order to ensure a continuous urine flow. The infusion
Figure 3 Relationships between (a) the BP and HR slopes of the cardiopulmonary reflex, (b) the gain ($G$) of the baroreceptor reflex and the BP slope of the cardiopulmonary reflex, and (c) the gain ($G$) of the baroreceptor reflex and the HR slope of the cardiopulmonary reflex.

Table 1: Resting parameters of rabbits during control infusion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>2.81 ± 0.12</td>
</tr>
<tr>
<td>GFR (ml·min$^{-1}$·kg$^{-1}$)</td>
<td>3.51 ± 0.32</td>
</tr>
<tr>
<td>ERPF (ml·min$^{-1}$·kg$^{-1}$)</td>
<td>10.29 ± 9.82</td>
</tr>
<tr>
<td>Na$^+$ clearance (µEq·min$^{-1}$·kg$^{-1}$)</td>
<td>4.65 ± 0.98</td>
</tr>
<tr>
<td>Li$^+$ clearance (mEq·min$^{-1}$·kg$^{-1}$)</td>
<td>1.00 ± 0.09</td>
</tr>
<tr>
<td>Urine volume (ml/min)</td>
<td>0.57 ± 0.07</td>
</tr>
</tbody>
</table>

Table 1. As in a previous study [2], the saline load produced a significant natriuresis within 30 min in the rabbits, which had a gain of their cardiac arterial baroreflex of more than 5 beats·min$^{-1}$·mmHg$^{-1}$, reaching peak values by 60–90 min of the start of the infusion. This was accompanied by an increase in urine volume, lithium clearance and effective renal plasma flow. The rates of sodium and lithium excretion and of renal plasma flow were much lower in rabbits with cardiac baroreflex sensitivity below 3.5 beats·min$^{-1}$·mmHg$^{-1}$. The saline infusion did not increase MAP in any of the rabbits. Regression coefficients were calculated between each of the values for sodium, lithium and PAH clearances 60–90 min after the start of the saline infusion, and the slopes of the BP and HR responses to PBG and the sensitivity of the cardiac arterial baroreflex. The relationships between sodium, lithium and PAH clearances and cardiopulmonary reflex sensitivity (MAP/dose of PBG) are shown in Figure 4. The Spearman (rho) correlation coefficients for these relationships are listed in Table 2 together with those derived from the slope of HR/dose of PBG relationship. Significant positive correlations (one-tailed) were found between the amounts of sodium, lithium and PAH excreted during the 60–90 min after the start of the saline infusion and each of these measures of cardiopulmonary baroreceptor sensitivity.

**DISCUSSION**

The major new finding in this study is that normotensive rabbits which show a delayed sodium excretion and fail to increase their renal blood flow in response to a saline infusion, have an impairment in the regulation of their cardiopulmonary baroreceptors. The cardiovascular response to chemical stimulation of these baroreceptors with PBG, a 5-hydroxytryptamine type 3 receptor agonist, was measured in conscious rabbits differing widely in the sensitivity of their arterial baroreceptors. The magnitude of the fall in BP as a function of the dose of PBG also varied widely among rabbits. The slope of the relationship of the fall in BP or HR to the dose of PBG was taken as a measure of the sensitivity of the cardiopulmonary baroreceptors to chemical stimulation.
Baroreflex sensitivity and sodium excretion

Figure 4 Relationships between the BP slopes of the cardio-pulmonary reflex and (a) Na\(^+\) clearance during 60–90 min of the infusion, (b) Li\(^+\) clearance during 60–90 min of the infusion, (c) effective renal plasma flow (PAH clearance) during 60–90 min of the infusion

Each of these measures was significantly correlated to the sensitivity of the arterial baroreceptors in 25 rabbits of both sexes.

Spontaneously hypertensive rats (SHR), which have impaired arterial baroreceptor sensitivity, have been shown to exhibit weaker cardiovascular responses to PBG than the normotensive Wistar-Kyoto strain [18], indicating a deficit in their cardiopulmonary reflex regulation. This finding is at variance with that of Ricksten and Thoren [19] who reported that the reflex sympathetic inhibition of cardiopulmonary baroreceptors by a 10% blood volume expansion was greater in SHR than in Wistar-Kyoto rats, even though their arterial baroreflex sensitivity was lower. The volume load caused a greater increase in the left atrial pressure in SHR than in the normotensive rats, which augmented cardiopulmonary-mediated inhibition of renal sympathetic nerve activity. This was probably due to a combination of decreased venous capacity and cardiac hypertrophy in the SHR, and a lower cardiac baroreflex sensitivity. When the hypertrophy was abolished by treatment with an angiotensin-converting enzyme inhibitor, perindopril, the vagal component of the cardiac arterial baroreflex increased significantly [20].

It is unlikely that the lower sensitivity of arterial and cardiopulmonary baroreceptors that was seen in some of the rabbits in the present study was due to structural changes in the heart or blood vessels since they were all normotensive. Moreover, the fact that SHR had a lower cardiovascular response to PBG than Wistar-Kyoto rats [18] suggests that structural changes in the heart and vasculature have less effect on the reaction of cardiopulmonary baroreceptors to chemical than to mechanical stimulation.

The site of the abnormality in baroreceptor regulation in the rabbits is not known. If it is not located in the baroreceptors themselves, it may be in the central nervous system, the efferent pathways or end organs. This question was addressed in Dahl salt-sensitive rats [21], which have several similarities to the rabbits with low baroreflex sensitivity. Both show impairments in arterial and cardiopulmonary baroreceptor sensitivity before hypertension develops [22, 23], and excrete a saline load more slowly than the respective salt-resistant animal [24]. It was found that the differences in sympathetic responses and HR in Dahl salt-sensitive and Dahl salt-resistant rats were still present after electrical stimulation of the afferent vagus, but not of the efferent nerves. This showed that there was an abnormality in central nervous system processing of baroreceptor information, in addition to an alteration in the baroreceptors themselves in Dahl salt-sensitive rats. It remains to be determined whether the abnormality in baroreflex sensitivity in the rabbits results from altered activity at this central nervous system site.

In response to a mild saline infusion, which did not increase MAP, marked differences were seen in the

©1998 The Biochemical Society and the Medical Research Society
natriuretic and haemodynamic responses in the rabbits, as reported previously [2,25]. Significant correlations were found between the sensitivity of cardiopulmonary baroreceptors as measured by the response to PBG and the excretion of lithium (a marker of proximal tubule reabsorption) and of PAH (a marker of effective renal plasma flow) induced by plasma volume expansion with saline. Both renal blood flow and lithium excretion are directly dependent on renal sympathetic nerve activity [8,26]. This in turn is strongly inhibited by activation of cardiopulmonary baroreceptors [27]. The correlation between the sensitivity of these receptors and the degree of sodium excretion was less pronounced, probably because the latter is influenced by other factors including hormones and local factors operating in the kidney [28]. This could also explain why sodium excretion was significantly increased by renal denervation, but the differences in the rate of natriuresis in rabbits with high and low baroreflex sensitivity remained [3].

A genetic impairment in the sensitivity of cardiopulmonary baroreceptors also occurs in human subjects and precedes the development of hypertension. This was seen in the depressed response to changes in cardiac filling in subjects with borderline hypertension [29] and in the delay in sodium excretion in young, normotensive men with a family history of hypertension after an acute saline infusion [30]. Thus, rabbits with genetic impairments in the regulation of their arterial and cardiopulmonary baroreceptors show similar abnormalities in their haemodynamic and excretory response to salt to these human subjects. These rabbits may therefore be an appropriate model for salt-sensitive hypertension in humans.

REFERENCES


Received 20 March 1998/11 June 1998; accepted 15 July 1998