INTRODUCTION

The presence of the calcium receptor on the cells surface (1) and the discovery of the compounds that might activate (2, 3) or inhibit (4) this receptor created the possibility of pharmacological correction of parathyroid hormone (PTH) secretion. Moreover, it was recently indicated that calcilytic NPS 2143 induces hypertension in rats. This study tested whether the increase of mean arterial blood pressure (MAP) induced by NPS 2143 administration is mediated by calcium channel and angiotensin II type1 (AT1) receptor activity. Wistar rats were anaesthetized with Thiopental i.p. and infused i.v. with saline supplemented with the anaesthetic. Blood pressure was monitored continuously in the carotid artery. Effects of NPS 2143 administered i.v. as bolus on MAP in the presence and absence of felodipine and losartan were investigated. Both, felodipine and losartan pretreatment provoked a persistent ΔMAP decrease by 18±3 and 14±3 mmHg, respectively. Infusion of NPS 2143 at 1 mg/kg b.w. confirmed hypertensive activity of calcilytic and increased blood pressure for 21±4 mmHg. In contrast, administration of NPS 2143 in felodipine as well as in losartan pretreated rats did not change ΔMAP as compared to felodipine/control and losartan/control groups, respectively. Our study indicated that both the blockade of calcium channels and the AT1 receptor activity prevented the hypertensive effect of calcilytic NPS 2143. This finding might be particularly important in understanding the mechanisms that mediated blood pressure changes related to the activity of calcium receptor.

Key words: calcium receptor, felodipine, losartan, angiotensin II, mean arterial blood pressure

MATERIALS AND METHODS

Animals

Male Wistar rats, weighing 200 to 250 g, were purchased from the Animal House of Mossakowski Medical Research Centre of the Polish Academy of Sciences, Warsaw, Poland. Rats were kept at constant room temperature (20°C) and humidity (70%) under...
12-h dark/light cycles. All experiments were approved by The Local Ethical Committee on Animal Experiments. The animals were fed a commercial rodent chow (Labofeed-B, Poland) and tap water ad libitum. On the day of the experiment, rats were anesthetized by the intraperitoneal injection of Thiopental at the dose 40 mg kg\(^{-1}\) b.wt. and kept under anesthesia by the infusion supplemented with Thiopental at the dose 30 µg kg\(^{-1}\) min\(^{-1}\), till the end of the experiment. The animals were placed on a heated table, and the body temperature was maintained between 36°C and 37°C. Thyroparathyroidectomy (TPTX) by heat cauterisation in some groups and tracheostomy was performed. Catheters were inserted into the carotid artery for pressure monitoring, into a jugular vein for infusions, and into the bladder for freely diuresis. Following all surgical procedures, a two-hour recovery period was allowed to establish a steady state. During the first hour of this period, rats were infused with 4% albumin in isotonic saline at the rate of 4.8 ml h\(^{-1}\). This infusion was then replaced by isotonic saline and maintained to the end of experiment. Blood pressure was monitored continuously.

**Effect of NPS 2143 on blood pressure in the presence and absence of thyroparathyroidectomy (TPTX)**

After 60 min of saline infusion, the NPS 2143 dissolved in 15% cyclodextrin (Sigma) at the dose 1 mg kg\(^{-1}\) b.wt. was infused in intact (n=5) and TPTX (n=4) rats. In control groups vehiculum alone was injected through venous catheter as a bolus in intact (n=4) and TPTX (n=5) rats. The time of administration of NPS 2143 or vehiculum administration was assumed as ‘time 0’.

**Effect of NPS 2143 on blood pressure in rats pretreated with losartan**

Rats were additionally infused with felodipine (Sigma) 60 µg kg\(^{-1}\) h\(^{-1}\), beginning 60 min prior to time 0, until the end of the experiment. At the time 0, the NPS 2143 (n=9) dissolved in 15% cyclodextrin (Sigma) at the dose 1 mg kg\(^{-1}\) b.wt. or vehiculum alone (n=8) were injected through venous catheter as a bolus.

**Effect of angiotensin II on blood pressure in rats pretreated with losartan**

Rats were additionally pretreated with losartan as a bolus, 20 mg kg\(^{-1}\) b.wt., 40 min before time 0. At the time 0, the NPS 2143 (n=5) dissolved in 15% cyclodextrin (Sigma) at the dose 1 mg kg\(^{-1}\) b.wt. or vehiculum alone (n=5) were injected through venous catheter as a bolus.

**Effect of angiotensin II on blood pressure in rats pretreated with felodipine**

Rats were additionally infused with felodipine 60 µg/kg/h, beginning from 60 min prior to time 0, until the end of experiment. At the time 0, the infusion was supplemented with angiotensin II 3 µg/kg/h (n=5). The control group of rats was infused with angiotensin II alone (3 µg/kg/h), beginning from time 0 (n=4).

**Measurements and statistical calculations**

Arterial blood pressure was monitored directly and sampled continuously at 100 Hz, as we described previously (5), using BIOPAC Systems Inc., Model MP 100 (Goleta, CA). The results of blood pressure measurements were processed with the help of the ACQKnowledge (Goleta, CA) measurement system. They were selected, scaled, and filtered in order to remove accidental signal disturbances. The recorded time domain transient data have been presented as graphs with the help of Matlab Code (MathWorks, Inc., Natic, MA).

Statistical analysis of variances of mean arterial blood pressure (MAP) after NPS 2143 or vehicle administration was performed for ∆MAP, calculated as a difference between MAP measured before time of calcifytic or vehicle administration (time 0) and MAP of sequential readings after that time, as we described previously (5). This allowed for direct comparison of responses to treatment between Groups when baselines differed. Data obtained after NPS 2143 or vehicle treatment and in the presence or the absence of felodipine and losartan were analysed by ANOVA with repeated measures, using Statistica StatSoft software (StatSoft, Inc., Tulsa, OK). In the case of significant changes, post-hoc comparisons were performed using Bonferroni and Duncan tests. A value of p<0.05 was considered statistically significant.

Statistical calculations for the effect of felodipine and losartan on blood pressure prior to NPS 2143 administration were performed using Student’s t paired test. Significance was designated as p<0.05.

**RESULTS**

Thiopental was produced by Sandoz GmbH, Austria. Losartan (Cadila Healthcare Ltd., India) was a gift from BIOFARM, Poland. NPS 2143 was synthesized according to the method of DelMar et al. (13) and Jirgensons et al. (14), in the Department of Organic Chemistry, Medical University of Gdansk, as was documented previously (8). All other reagents were purchased from Sigma-Aldrich, Poland.

In the absence of felodipine or losartan administration of calcifytic NPS 2143 increased blood pressure as compared to control animals (p<0.006) and remaining experimental groups (p<0.001), (Fig. 1, 3). Administration of NPS 2143 in TPTX rats did not change blood pressure (Fig. 2).

Results of the experiments determining whether the bolus injection of NPS 2143 affected the blood pressure during felodipine infusion in rats are presented in Fig. 1. Panel a) shows results of direct measurements; panel b) shows ∆MAP, i.e., the difference in MAP between sequential measurements and MAP at the moment of NPS 2143 administration (time 0). Pretreatment with felodipine, the calcium channel blocker, induced a significant (p<0.001) decrease of ∆MAP as compared to initial period (Table 1). The average decrease reached 17 mmHg before administration of the NPS compound. Beginning from the moment of NPS 2143 administration, the MAP in the felodipine/control group remained constant in the absence of felodipine and losartan administration on blood pressure.

**Table 1. Effect of felodipine and losartan administration on blood pressure in rats.**

<table>
<thead>
<tr>
<th>Time of experiment (min)</th>
<th>Felodipine n=17</th>
<th>Losartan n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td>-60</td>
<td>106 ± 3</td>
<td>-</td>
</tr>
<tr>
<td>-40</td>
<td>-</td>
<td>114 ± 3</td>
</tr>
<tr>
<td>0</td>
<td>89 ± 2*</td>
<td>100 ± 4*</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE. MAP, mean arterial pressure; n, number of animals; *, p<0.001 vs. control period calculated using Student’s t paired test.
Similarly, the administration of calcilytic NPS 2143 during felodipine infusion did not change $\Delta$MAP compared to the control and felodipine/control groups (panel b).

The effect of NPS 2143 on the blood pressure in the animals pretreated with losartan, angiotensin II type 1 receptor (AT1) blocker, is presented in Fig. 3. Administration of losartan induced a significant fall in $\Delta$MAP, by about 14 mmHg (Table 1). In losartan/control and losartan/NPS 2143 groups, beginning from time of NPS 2143 administration, $\Delta$MAP did not change significantly as compared to the control group (Fig 3, panel b).

Data showing the degree of prevention of hypertensive effect exerted by angiotensin II during felodipine infusion in our experimental conditions is presented in Fig 4, panel a. Infusion of angiotensin II increased within 7 min blood pressure by 34±5 mmHg, which remained unchanged for subsequent 50 min of experiment. On the other hand, in animals pretreated with felodipine, angiotensin II within 5 min increased blood pressure by 16±1 mmHg only, which was followed by a decrease down to 6±3 mmHg at 60 min of experiment (Fig. 4, panel b).

**DISCUSSION**

In the present investigation the new observation is the lack of the hypertensive effect of NPS 2143 in the presence of felodipine - calcium channel inhibitor and in the presence of losartan - AT1 receptor antagonist. The study also confirmed previously documented hypertensive effect of calcilytic NPS 2143 (8) in rats that were not pretreated with felodipine or losartan.

Experiments were performed under *in vivo* conditions since it was found that the hypertensive effect of NPS 2143 was observed only in the presence of parathyroid glands (8). This observation has been confirmed also in our present study. It is hypothesized that calcilytic administration, through mechanisms similar to those that control PTH secretion, might mediate the secretion of other substances produced by parathyroid glands. It is well documented that parathyroid glands secrete parathyroid hypertensive factor (PHF), which was confirmed in patients with primary hyperparathyroidism and hypertension (15) and in...
hypertensive rats (SHR) (16, 17). Moreover, it was shown that PHF markedly increased Ca°° influx to rat tail arteries (18). This suggests that hypertensive effect of NPS 2143 might be mediated by increased influx of Ca°° to arterial smooth muscle cells, resulting in arterial contraction. [Ca°°], and vascular contractility are closely dependent on the activity of calcium channels and AT1 receptors present in the cell membrane of the vascular smooth muscle cells (9, 11). Therefore, blockade of calcium channels and AT1 receptors of vascular system may diminish hypertensive effect of calcilytic.

The blood pressure changes are presented as direct measurements and as ΔMAP. The ΔMAP was calculated as a difference in MAP between the sequential measurements and the MAP value in the time of application of tested compound. Statistical comparisons of blood pressure changes were carried out for ΔMAP. This approach enabled a direct comparison of responses to treatment between experimental groups even if the baselines differed.

We used felodipine as a calcium channel blocker regarding its arteriolar vasoselectivity and almost lack of cardiodepressant activity (11, 12). Losartan was used as AT1 receptor antagonist (9). The optimal doses of both factors were established experimentally (data not shown). Pretreatment with felodipine and losartan lowered blood pressure in rats. The decrease in the absolute values approximated the rise in blood pressure after NPS 2143 administration and reached 18 and 14 mmHg, respectively. Moreover, in the control/felodipine and control/losartan animals the blood pressure remained roughly stable after time 0 of experiment. Therefore, the changes of blood pressure observed after NPS 2143 injection in rats pretreated with felodipine and losartan may be interpreted as the effect of calcilytic alone.

![Graph](image.png)

**Fig. 2.** Effect of NPS 2143 administration: (a) on the mean arterial pressure (MAP) and (b) on ΔMAP (calculated as a difference of MAP between sequential measurements and a value of MAP at the moment of NPS 2143 or vehicle administration) in thyroparathyrodecomized (TPTX) rats. Each point represents the mean value of MAP or ΔMAP from: TPTX/NPS 2143 (n=4) and TPTX/control (n=5) experimental groups. Comparisons were made using ANOVA with repeated measures. Significance was designated as p<0.05.
The effect of NPS 2143 on ∆MAP in the presence of felodipine and losartan did not differ from that observed in the control, felodipine/control and losartan/control rats. This indicated that under in vivo conditions administration of felodipine as well as losartan totally abolished hypertensive effect of calcilytic in rats. Therefore, it may be presumed that calcium receptor dependent secretion of a hypertensiogenic factor from parathyroids affected vascular system preferentially via increased influx of Ca²⁺ to arterial smooth muscle cells.

Recently it was reported that dichlorobenzamil efficiently activated Ca²⁺-activated K⁺ channels in fresh isolated mouse aortic smooth muscle cells (19). Blockade of the hypertensive effect of NPS 2143 by felodipine may suggest that a parathyroid substance directly effected calcium channels of vascular smooth muscle. This seems to be a questionable hypothesis, however, it was shown that PHF inhibits voltage-gated K⁺ channels in vascular smooth muscle cells (20). Therefore, one may not exclude that the T and L-type calcium channels could be activated due to the cell depolarization. This possibility stays in agreement with the present findings indicating that OR-1896, a novel inodilator, is acting mainly via calcium sensitization and opening of ATP-sensitive K⁺ channels (21).

It is accepted that losartan decreases intracellular Ca²⁺ concentration due to inhibition of intracellular mobilization of Ca²⁺, as well as extracellular Ca²⁺ influx, while felodipine inhibits extracellular influx of Ca²⁺ only (22). Therefore, one may expect only partial abolishment of hypertensive effect of NPS 2143 in felodipine pretreated rats. In our experiments felodipine as well as losartan completely prevented hypertensive activity of NPS 2143. For that reason, the extent of decrease of hypertensive activity of angiotensin II during felodipine infusion

Fig. 3. Effect of NPS 2143 or vehicle (control) administration in the presence and absence of losartan: (a) on the mean arterial pressure (MAP) and (b) on ∆MAP (calculated as the difference of MAP between sequential measurements and a value of MAP at the moment of NPS 2143 or vehicle administration) in rats. Each point represents the mean value of MAP or ∆MAP from: NPS 2143 (n=5), control (n=4), losartan/control (n=5), losartan/NPS 2143 (n=5) experimental groups. Comparisons were made using ANOVA with repeated measures, Bonferroni and Duncan test. Significance was designated as p<0.05.
was additionally tested. In our experimental conditions, felodipine decreased the hypertensive effect of angiotensin II by approximately 55%. Therefore, it seems likely that among mechanisms mediating hypertensive effect of NPS 2143 activity of the calcium channels plays more important role than other pathways involved in [Ca²⁺] increase.

Administration of NPS 2143 increases the plasma PTH concentration in rats (3, 8). It has been observed that the single doses of 1-34 PTH (23-25) or 1-84 PTH (25) transiently decreased blood pressure in the intact (25) as well as in thyroparathroidectomized (23) animals. In these experiments, decrease of blood pressure was observed immediately after PTH injection. Therefore, the possible influence of increased PTH on vascular system could be considered as decreasing blood pressure in rats. In our study, in the absence of felodipine and losartan, application of calcilytic resulted in the increase of blood pressure. Consequently, under in vivo conditions, the direct effect on vessels in rats treated with NPS 2143 may reflect the final effect of both, hypertensive factor and PTH.

Several investigators have reported that calcium receptor is expressed in many cardiovascular tissues, including human aortic endothelial cells (26) and rat cardiomiocytes (27). Calcium receptor has been also detected in homogenates of whole vessels from rat subcutaneous small arteries (28) and aortic smooth muscle cells (29). Nevertheless, there were also discordant reports on the presence of calcium receptor in the vascular smooth muscle cells (30, 31). So far, there are no reports on calcilytic effect on the vessel contractility ex vivo, but the possibility of direct influence

Fig. 4. Effect of angiotensin II administration in the presence and absence of felodipine: (a) on the mean arterial pressure (MAP) and (b) on ΔMAP (calculated as a difference of MAP between sequential measurements and a value of MAP at the moment of angiotensin II administration) in rats. Each point represents the mean value of MAP or ΔMAP from: angiotensin II (n=4) and felodipine/angiotensin II (n=5) experimental groups. Comparisons were made using ANOVA with repeated measures, Bonferroni and Duncan test. Significance was designated as *p<0.05.*
of NPS 2143 on blood vessels is not excluded. Recently, results of the investigation regarding vascular effect of calcium receptor agonist, calcimimetic AMG 073, have been published. Experiments performed on isolated rat aortas indicated relaxation of vessels precontracted with an activator of voltage-dependent calcium channel (32). Although results of our studies suggest that calcium receptor present on the parathyroid gland mediates hypertensive effect of calcilytic, other mechanism/s, such as direct effect on vessels, may contribute this effect as well.

In conclusion, our experiments indicate that pretreatment with felodipine and losartan prevents the hypertensive effect of calcilytic NPS 2143 in rats. Although, there are several mechanisms of this effect, our data suggest the high significance of the pathways that increase [Ca²⁺] via extracellular influx. Our observation might be of particular importance in understanding the mechanisms of hypertension in patients with primary and secondary hyperparathyroidism.

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Conflict of interests: None declared.

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