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Calcimimetic NPS R-568 Induces Hypotensive Effect in Spontaneously Hypertensive Rats

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Background: The discovery of calcium receptors and calcimimetics created the possibility of "pharmacologic parathyroidectomy" (phPTX), which decreased secretion of parathormone (PTH). Parathyroid glands of spontaneously hypertensive rats (SHR) and of patients with primary hyperparathyroidism and hypertension secrete parathyroid hypertensive factor (PHF). Parathyroidectomy decreases blood pressure in these rats and in patients. The present study determined whether phPTX induced by calcimimetics decreases mean arterial blood pressure (MAP) in hypertensive rats.

Methods: Hypertensive SHR and normotensive Wistar Kyoto (WKY) rats were used. Clearance experiments were performed and the effect of 1 mg/kg body weight (given intravenously) synthesized NPS R-568 (NPS) on MAP in the presence or absence of thyroparathyroidectomy (TPTX) was monitored.

Results: The success phPTX and TPTX were proven by a significant decrease in plasma Ca^{2+} concentration and a decrease in urinary fractional phosphate excretion (FE Pi). The administration of NPS significantly decreased blood pressure in SHR versus SHR/control: Δ(0–50 min of experiment) MAP -16.5 ± 2.5 mm Hg *v* -3.2 ± 1.5 mm Hg ($P < .002$). The TPTX decreased blood pressure in SHR versus SHR/control and was not different versus SHR/TPTX/NPS (ΔMAP: -10.2 ± 1.6 mm Hg *v* -3.2 ± 1.5 mm Hg ($P < .01$) and *v* -8.3 ± 2.2 mm Hg (P = not significant). In normotensive WKY rats application of NPS did not reach significance in ΔMAP: -6.7 ± 1.8 mm Hg *v* -2.6 ± 2.8 mm Hg (P = not significant) in WKY/control. The TPTX lowered blood pressure in WKY versus WKY/control and remained unchanged versus WKY/TPTX/NPS (ΔMAP: -11.3 ± 1.7 mm Hg *v* -2.6 ± 2.8 mm Hg ($P < .04$) and *v* -11.4 ± 2.6 mm Hg (P = not significant).

Conclusions: We conclude that phPTX with NPS R-568 is responsible for a decrease of MAP in SHR. Am J Hypertens 2005;18:364–371 © 2005 American Journal of Hypertension, Ltd.

Key Words: Pharmacologic parathyroidectomy, calcium receptor, hypertension, parathyroid hormone, primary hyperparathyroidism.

Primary hyperparathyroidism frequently results in the development of hypertension but the mechanism of this observation is still unknown.[1,2] It was reported that parathyroidectomy of hyperactive parathyroid glands would decrease blood pressure (BP) in humans.[3–5] A similar phenomenon was observed after parathyroidectomy in spontaneously hypertensive rats (SHR).[6,7]

These observations lead to the discovery of the hypertensive factor originating from the parathyroid glands—parathyroid hypertensive factor (PHF). The existence of this factor was confirmed in SHR with congenital hypertension[8,9] and in patients with primary hyperparathyroidism accompanied with hypertension.[10] Parathyroidectomy performed in patients with primary hyperparathyroidism and hypertension decreases the activity of PHF and decreases BP in these patients.[11]

The discovery of calcium receptors and calcimimetics,[12,13] compounds that by activation of calcium receptor of parathyroid cells decrease parathormone (PTH) secretion, make possible the performance of "pharmacologic parathyroidectomy" in rats[14,15] and in humans.[16,17] Therefore, the possibility exists that pharmacologic parathyroidectomy in hypertensive SHR rats with calcimimetics induces, similarly to decreased PTH secretion, a decrease in PHF secretion, subsequently lowering BP. At present, there have not been available data regarding the influence of calcimimetics on BP in hypertensive rats or hyperten-

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sive patients with primary hyperparathyroidism. Therefore, we administered the calcimimetic, NPS R-568, which reduces PTH secretion in rats[18,19] and in patients with primary and secondary hyperparathyroidism.[16,20]

Our study was performed to compare the effect of the calcimimetic NPS R-568 and thyroparathyroidectomy (TPTX) on mean arterial BP in hypertensive SHR compared to its normotensive control, the Wistar Kyoto (WKY) rat.

Methods

Clearance Experiments

Male SHR and WKY rats (Polish Mother's Memorial Hospital, Research Institute, Lódź, Poland), weighing 200 to 280 g, were used. The animals were fed a commercial rodent chow and tap water ad libitum. Rats were anesthetized by intraperitoneal injection of thiopental at the dose 40 mg/kg body weight. The animals were placed on a heated table and body temperature was maintained between 36° and 37°C. Thyroparathyroidectomy by heat cauterization in some animals and tracheostomy in all experimental groups were performed. Catheters were inserted into the carotid artery and jugular vein for pressure monitoring, blood sampling, and infusions. The urinary bladder was cannulated for urine collection. Blood pressure was constantly monitored. Glomerular filtration rate (GFR) was measured as [^{3}H]inulin clearance.

After all surgical procedures, including TPTX, a 2-h recovery period was allowed to establish steady state. For the first hour, rats were infused with 4% albumin in isotonic saline at rate of 5.6 mL/h. This infusion was then replaced by isotonic saline at the same rate. For the next hour, an intravenous bolus of [^{3}H] inulin (Amersham Biosciences, Piscataway, NJ), 3 μCi/250 g body weight, was given and the infusion of saline supplemented with [^{3}H]inulin (0.02 μCi/min) was started and maintained until the end of the experiment.

Experimental Groups

Ten groups of rats were studied according to the following protocols:

Group 1: SHR/NPS ($n = 6$) After 30 min of [^{3}H]inulin infusion, the NPS R-568 (NPS) was dissolved in 15% cyclodextrin (Sigma-Aldrich, Poznan, Poland) at a dose of 1 mg/kg body weight through the venous catheter, administered as a bolus. The time of administration of NPS was assumed as time 0. Blood samples were taken at the midpoint of 10 min urine collections at 5 min before and at 30, 60, and 90 min after administration of the test agent.

Group 2: SHR/control ($n = 5$) This procedure was similar to that in group 1. Only 15% cyclodextrin was administered intravenously.

Group 3: SHR–TPTX/NPS ($n = 5$) and Group 4: SHR–TPTX/control ($n = 4$) Before tracheostomy, each rat underwent TPTX. All other procedures were the same as in groups 1 and 2 (group 3 corresponds to group 1, and group 4 corresponds to group 2).

Group 5: WKY/NPS ($n = 4$) and Group 6: WKY/control ($n = 4$) The same protocols as in groups 1 and 2 were performed on WKY rats.

Group 7: WKY–TPTX/NPS ($n = 5$) and Group 8: WKY–TPTX/control ($n = 4$) The same protocols as in groups 3 and 4 were performed on WKY rats.

To measure the effect of NPS R-568 on plasma PTH concentration, two additional groups of rats were included:

Group 9: SHR/NPS ($n = 6$) After a 2-h recovery period, the NPS (as in group 1) was administered as a bolus. Blood samples (1.5 mL) for assay of PTH concentration were taken 10 min before and at 15, 30, and 60 min after administration of NPS. To prevent excessive blood loss, immediately after separation of plasma, the sediment of red cells was rinsed with saline and reinfused as the erythrocyte concentrate.

Group 10: SHR/control ($n = 4$) This procedure was similar to that in group 9. Only 15% cyclodextrin was administered intravenously.

Measurements and Calculations

Total radioactivities of blood and urine samples were counted on liquid scintillation counter Wallac 1409 (LKB, Sweden). Phosphate concentrations in plasma and urine were determined according to the adapted method of Fiske and SubbaRow.[21] The plasma pH and ionized calcium [Ca^{2+}] and sodium [Na^+] concentrations were measured using AVL 988-4 Ca^{2+}/pH analyzer (AVL, Vienna, Austria). Plasma PTH concentration was determined with rPTH radioimmonoassay kit (Pennisula Laboratories, Inc., San Carlos, CA). Values are presented as means $\pm$SE. Comparisons were made using the Student t test. Significance was designated as $P < .05$.

Arterial BP was monitored directly and sampled continuously at 100 Hz, using BIOPAC Systems Inc., model MP 100 (Goleta, CA). The BP measurements were selected, scaled, and filtered to remove the accidental signal disturbances with the help of ACQKnowledge (Goleta, CA). The recorded time domain transient data have been presented as graphs in smoothed form. Smoothing has been carried out by means of polynomial approximation method, applying polynomials of degree 16, that fits the data sets in a least-square sense, with the help of Matlab Code (MathWorks, Inc., Natick, MA).

Statistical analysis of variance of mean arterial BP (MAP) were performed for ΔMAP, calculated as the difference of MAP between sequential measurements and time 0 min of the experiment for each group. This allowed for direct comparison of the responses to treatment between groups when baselines differed. Data were analyzed by ANOVA with repeated measures, using Statistica StatSoft software (StatSoft, Inc., Tulsa, OK), for SHR and