Effects of Salt Loading on Blood Pressure in Mice Lacking the Prostanoid Receptor Gene

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Background This study examined whether targeted disruption of the genes for the prostacyclin receptor (IP) or the thromboxane A2 receptor (TP) confers a susceptibility to salt-dependent hypertension.

Methods and Results Eight female IP- or TP-deficient mice were examined. Baseline systolic blood pressure (SBP) did not differ between TP(–/–) and TP(+/+), but was significantly lower in the IP(–/–) group than in the IP(+/+). With a high salt diet, SBP in IP(–/–) gradually increased. In contrast, SBP in the IP(+/+) group remained unchanged.

Conclusions The prostacyclin receptor may participate in the maintenance of baseline BP. With salt loading, BP adaptation may take place, at least in part, via IP mediated signals. (Circ J 2005; 69: 124–126)

Key Words: Blood pressure; Gene targeting; Prostacyclin (IP) receptor; Salt sensitivity; Thromboxane A2 (TP) receptor

Prostanoids are a group of bioactive lipids working as local mediators and include the D, E, F and I types of prostaglandins (PGs) and thromboxanes (TXs). Prostacyclin and TXA2 are thought to be important for systemic vascular homeostasis and thrombogenesis. Renal prostanoids have important local functions including control of glomerular microcirculation and tubular handling of sodium. The involvement of prostanoids in the regulation of body fluid and blood pressure (BP) in response to salt loading has been reported; but the mechanism of BP control is multifactorial and not yet completely elucidated, nor is it fully understood which type of prostanoids participate in BP control. To address these points, we investigated whether targeted disruption of the genes for the prostacyclin receptor (IP) or TXA2 receptor (TP) confers susceptibility to salt-dependent changes in BP.

Methods

Derivation of Mutant Mice

We disrupted the mouse genes encoding IP5,6 and TP (unpublished data) and established 2 lines of embryonic stem cells. In brief, chimeric mice were generated from each line. The mice were backcrossed with C57BL/6 mice and the resulting heterozygous litter mates IP(+/-) and TP(+/-) were bred to produce homozygous mice. Reverse transcriptase polymerase chain reaction confirmed the absence of IP or TP transcripts in the kidneys of homozygous mice. The presence of IP or the thromboxane A2 receptor (TP) confers susceptibility to salt-dependent changes in BP.

Protocol

Eight female IP- (12–14 weeks of age) or TP- (12 weeks of age) deficient mice were placed in metabolic cages for 9 weeks. The initial body weights of the IP(–/–), IP(+/+), and TP(–/–), and TP(+/+) mice were 31.2±2.3 g, 30.0±3.2 g (NS vs IP(–/–)), 24.0±1.6 g, and 24.2±3.4 g (NS vs TP(–/–)), respectively. These mice were fed a diet with 0.3% NaCl (low salt) after weaning until the experiment, after which they were fed an 8% NaCl (high salt) diet for 3 weeks and then fed the low-salt diet again for another 3 weeks (Figs 1,2). Eight wild-type age-matched controls underwent the same protocol. After the high-salt diet, all the mice were fed with the low-salt diet again for 2 weeks for BP measurement, and then all the mice were killed by decapitation for blood sampling.

Measurements

Basal BP and heart rate were measured in conscious animals by the tail-cuff method. The stable metabolites of TXA2 and prostacyclin in urine were measured by gas chromatography/mass spectrometry.

Results

The urinary excretion of TXB2, 2,3 dinor TXB2, 11-dehydro-TXB2, 6-keto-PGF1a, and 2,3 dinor 6-keto-PGF1a in TP(–/–) and IP(–/–) mice did not differ from that in their corresponding wild-type mice on the 0.3% salt diet. The baseline systolic BP (SBP) on week 20 determined by tail-cuff method did not differ between TP(–/–) and TP(+/+) mice (Fig 1), but was significantly lower in IP(–/–) than in IP(+/+) mice (p=0.005, Fig 2). Diastolic BP was recorded because of its inconsistency with the tail-cuff method. TP(–/–), IP(–/–), and the wild-type mice were then placed on the high-salt diet for 3 weeks. After 14 days, it was confirmed that the 8% NaCl diet was influencing urinary NaCl excretion, which did not differ among the TP(–/–), IP(–/–), and the corresponding wild-type mice (TP(–/–): from 0.39±0.1 in week 20 to 3.4±0.6 mmol/day in week 23; IP(–/–): 0.25±0.1 in week 20 to 3.3±1.5 mmol/day.
in week 23; TP(+/+): 0.41±0.1 in week 20 to 4.3±
0.7 mmol/day in week 23; IP(+/+): 0.28±0.1 in week 20 to
4.9±1.5 mmol/day in week 23 mmol/day). SBP in the
IP(−/−) mice increased significantly (from 119±6 in week
20 to 128±5 mmHg in week 23; p=0.005, Fig 2), but
remained unchanged in the IP(+/+), TP(−/−), or TP(+/+)
mice throughout the period of salt loading (Figs 1,2). After
the salt-loading period had finished, SBP in the IP(−/−)
mice returned to the baseline level with the low-salt diet
(Fig 2). Urinary excretion of TXA2 metabolites and prosta-
cyclin metabolites in the TP(−/−) mice did not differ from
that in the corresponding wild-type mice on the high-salt
diet for 3 weeks. However, the urinary excretion of prosta-
cyclin metabolites in the IP(−/−) mice increased significantly and was higher
than in the wild-type mice on the high-salt diet (IP(−/−): 4,700±1,500 ng/day; IP(+/+): 2,700±1,000 ng/day;
p<0.001). The values for blood urea nitrogen and serum
creatinine were not significantly different among all the
groups.

Discussion

Although there was a difference in body weight between
the IP(−/−) and TP(−/−) mice, which might be partly related
to the divergence in their genetic backgrounds and ages at
the time of the experiment, there were no major organ
abnormalities (data not shown) or growth disturbances in
either group of mice as previously reported. Renal function
at the endpoint was within normal limits and there were no
differences among all the experimental groups of mice.
Baseline SBP in the IP(−/−) mice was significantly lower
than that of the IP(+/+), which seems a remarkable finding
because prostacyclin is a vasodilator. Thus, our results
suggest that IP may participate in the maintenance of base-
line BP through mechanism(s) other than vasodilation.
Alternatively, the uneven genetic backgrounds of the
IP(+/+) and IP(−/−) mice may have in
fluence the difference in SBP at baseline. The urinary excretion of sodium
did not differ between the IP(+/+) and IP(−/−) mice, or the
TP(+/+) and TP(−/−) mice, during each experimental
period, confirming that sodium intake was equivalent in
each group. The urinary excretion of prostacyclin metabo-
lites in IP(−/−) mice increased significantly and was higher
than in the wild-type mice on a high-salt diet, demon-
strating that salt loading increases the renal production of
prostacyclin. Taken together, our results suggest that
prostacyclin is generated to counterregulate salt loading
and that the signal for BP regulation upon salt loading is at
least in part mediated through IP, the mechanism of which
remain to be further elucidated. Interestingly, it has been
previously demonstrated that targeted disruption of EP2
caused salt-sensitive hypertension, suggesting that vasodi-
lating prostaglandin receptors, including IP and EP2, may play
a common role in the regulation of systemic BP. The pres-
ent study does not address all the possible causes of salt-
sensitivity; however, as sodium retention (antiinatriuresis)
is a consequence of NSAID use in some patients\textsuperscript{8} we speculate that the IP(−/−) mice on a high-salt diet have impaired sodium and water excretion, which chronically leads to high SBP.

In conclusion, IP may participate in the maintenance of baseline BP and after salt loading, BP adaptation may take place, at least in part, via increased prostacyclin production and IP-mediated signals.

References


