Inactivation of Enhanced Expression of G\textsubscript{i} Proteins by Pertussis Toxin Attenuates the Development of High Blood Pressure in Spontaneously Hypertensive Rats

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Abstract—We have previously shown that the enhanced expression of G\textsubscript{i} proteins in spontaneously hypertensive rats (SHR) that precedes the development of high blood pressure may be one of the contributing factors in the pathogenesis of hypertension. In the present study, we demonstrate that the inactivation of G\textsubscript{i} proteins by intraperitoneal injection of pertussis toxin (PT, 1.5 μg/100 g body wt) into 2-week-old prehypertensive SHR prevented the development of hypertension up to 4 weeks and that, thereafter, it started to increase and reached the same level found in untreated SHR after 6 weeks. A second injection of PT after 4 weeks delayed the increase in blood pressure for another week. The PT-induced decrease in blood pressure in 6-week-old SHR was associated with a decreased level of G\textsubscript{i-2} and G\textsubscript{i-3} proteins in the heart, as determined by in vitro ADP ribosylation and immunoblotting. The decreased level of G\textsubscript{i} proteins was reflected in decreased G\textsubscript{i} functions. Furthermore, an augmentation of blood pressure to the same level in PT-treated SHR as found in untreated SHR was associated with enhanced expression and function of G\textsubscript{s}. These results indicate that the inactivation of G\textsubscript{i} proteins by PT treatment in prehypertensive SHR attenuates the development of hypertension and suggest that the enhanced levels of G\textsubscript{i} proteins that result in the decreased levels of cAMP and associated impaired cellular functions may be contributing factors in the pathogenesis of hypertension in SHR. (Circ Res. 2002;91:●●●●

Key Words: pertussis toxin ■ ADP ribosylation ■ G proteins ■ blood pressure ■ spontaneously hypertensive rats

Guanine nucleotide regulatory proteins (G proteins) are a family of GTP-binding proteins that play an important role in the regulation of a variety of signal transduction systems, including the adenylyl cyclase/cAMP system. The adenylyl cyclase system is composed of three components: receptor, catalytic subunit, and stimulatory (G\textsubscript{s}) and inhibitory (G\textsubscript{i}) guanine nucleotide regulatory proteins. The stimulation and inhibition of adenylyl cyclase by hormones are mediated by these two distinct G proteins (G\textsubscript{s} and G\textsubscript{i}, respectively), which couple the receptor to the catalytic subunit. The G proteins are heterotrimeric and are composed of α, β, and γ subunits. The α subunits bind and hydrolyze GTP and confer specificity in receptor and effector interactions. Molecular cloning has revealed four different forms of G\textsubscript{ai} resulting from the differential splicing of one gene and three distinct forms of G\textsubscript{ia} (G\textsubscript{ia-1}, G\textsubscript{ia-2}, and G\textsubscript{ia-3}) encoded by three distinct genes. All three forms of G\textsubscript{ia} (G\textsubscript{ia-1} to G\textsubscript{ia-3}) are implicated in adenylyl cyclase inhibition and in the activation of atrial K\textsuperscript{+} channels.

The adenylyl cyclase/cAMP system has been implicated in both the control of heart contractility and vascular smooth muscle tone. The levels of cAMP are regulated by G\textsubscript{s} and G\textsubscript{i} proteins. G\textsubscript{i} protein and associated adenylyl cyclase signaling has been shown to be implicated in a variety of cellular functions, including vascular permeability, salt and water transport, and catecholamine release, all of which play a key role in the regulation of blood pressure (BP). Alterations in the levels of G\textsubscript{s} proteins and cAMP levels that result in the impaired cellular functions lead to various pathological states, including hypertension. Several abnormalities in G-protein expression, adenylyl cyclase activity, and cAMP levels have been reported in cardiovascular tissues from genetic models (spontaneously hypertensive rats [SHR]) and different models of experimentally induced hypertensive rats. An increased expression of G\textsubscript{i} protein and G\textsubscript{i} protein mRNA in hearts and aortas from SHR and in hearts from doxycorticosterone acetate (DOCA)-salt hypertensive rats with established hypertension has been reported. On the other hand, the levels of G\textsubscript{ai} were shown to be unaltered in SHR but were decreased in DOCA-salt hypertensive rats. The increased levels of G\textsubscript{ai} were shown to be associated with hypertension and not with hypertrophy, whereas the decreased levels of G\textsubscript{ai} were associated with hypertrophy and not with hypertension. We have recently shown that the
Effect of in vivo PT treatment on development of high BP. Two-week-old SHR and WKY rats were injected intraperitoneally with PT (1.5 μg/100 g body wt) in 0.01 mol/L sodium phosphate buffer, pH 7.0, containing 0.05 mol/L NaCl (PT-treated) or vehicle (control WKY rats and control SHR), as described in Materials and Methods. A second injection of PT (1.5 μg/100 g body wt) was given at 6 weeks to one group and at 8 weeks to another group of PT-treated SHR and PT-treated WKY rats. BP was monitored weekly as described in Materials and Methods. Values are mean±SEM of 5 or 6 rats in each group. *P<0.05 and †††P<0.001 for WKY rats vs various groups; †P<0.05 and ††P<0.001 for SHR vs PT-SHR.

Materials and Methods

Materials

AS/7 and EC/2 antibodies and [α-32P]NAD+ were purchased from DuPont Canada. The C-atrial natriuretic peptide fragment 4-23 (C-ANP 4-23) was purchased from Peninsula Laboratories, and PT was purchased from DuPont Canada. The C-atrial natriuretic peptide fragment 4-23 was purchased from List Biochemicals. All other materials were purchased from commercial sources and were of the highest purity available.

Animal Treatment

Male SHR (2 weeks old) and age-matched Wistar-Kyoto (WKY) rats were purchased from Charles Rivers Canada (St-Constant, Quebec, Canada) and housed at the University of Montreal for 2 days. PT (1.5 μg/100 g body wt) or vehicle was injected into 2-week-old SHR and their age-matched WKY rats, respectively, as described earlier. A second injection of PT (1.5 μg/100 g body wt) was given to one group of PT-treated SHR at 6 weeks and to another group at 8 weeks. The BP was measured weekly up to 9 weeks by the tail-cuff method without anesthesia. The rats were euthanized by decapitation at 6, 8, and 9 weeks of age. The hearts and mesenteric arteries were dissected out, frozen immediately in liquid nitrogen, and stored at −80°C. All biochemical studies were performed in 6- and 8-week-old control and PT-treated SHR and WKY rats that received a single injection of PT or vehicle, respectively, at 2 weeks of age.

Preparation of Heart and Mesenteric Artery Particulate Fraction

Heart and mesenteric artery particulate fractions were prepared as described previously.

PT-Catalyzed ADP Ribosylation

ADP ribosylation of heart membranes by PT was performed as described previously. The heart membranes were solubilized with Lubrol PX (0.3%) at 25°C for 10 minutes and were centrifuged at 10,000g for 1 hour. The solubilized fractions were incubated in 25 mmol/L glycylglycine buffer, pH 7.5, containing 15 μmol/L [α-32P]NAD+ (20 μCi/mL), 0.4 mmol/L ATP, 0.4 mmol/L GTP, 15 mmol/L thymidine, 10 mmol/L diethiothreitol, and 0.1 mg/mL ovalbumin with or without PT (5 μg/mL) for 30 minutes at 30°C in total volume of 100 μL. The reaction was terminated by the addition of 20 μL stop mixture containing 5% SDS and 50% β-mercaptoethanol. The proteins were analyzed by SDS-PAGE and subsequently quantified as described previously.

Immunoblotting

Immunoblotting of Gα proteins in heart particulate fractions was performed using specific antibodies against different Gα proteins and the enhanced chemiluminescence kit (Amersham), as described previously.

Adenylyl Cyclase Assay

Adenylyl cyclase activity was determined by measuring [32P]cAMP formation from [α-32P]ATP, as described previously.

Data Analysis

Data are expressed as mean±SEM and were analyzed by ANOVA in conjunction with the Newman-Keuls test where applicable. Differences between groups were considered statistically significant at P<0.05.

Results

Effect of In Vivo PT Treatment on Development of High BP

The BP profile is shown in Figure 1. Mean arterial BP was not significantly different in 3-week-old SHR compared with age-matched WKY; however, BP started to increase from 4 weeks in SHR. On the other hand, PT-treated SHR did not show any increase in BP up to 6 weeks of age; thereafter, BP started to increase and reached the same level as that of
untreated SHR at 8 weeks. A second injection of PT at this time point again decreased the BP significantly but not to the control WKY level. However, a second injection of PT at 6 weeks into PT-treated SHR delayed further the increase in BP for another week. After that, it started to go up but was always lower than the BP of untreated SHR. On the other hand, PT treatment did not significantly affect the BP in WKY rats. PT (1.5 mg/100 g body wt) did not appear to have adverse effects on the health of animals in the study, because all rats treated with PT maintained or gained weight during the period of the studies (body weights were as follows: for WKY rats, 160 ± 7.8 g; for PT-treated WKY rats, 154 ± 7.4 g; for SHR, 150 ± 7.0 g; and for PT-treated SHR, 149 ± 3.9 g).

In addition, as reported earlier, the ratio of heart weight to body weight was not different in 6- and 8-week-old SHR compared with their age-matched WKY rats and was not affected by PT treatment (data not shown).

**Effect of In Vivo PT Treatment on PT-Catalyzed ADP Ribosylation of G Proteins**

The in vitro ADP ribosylation studies were conducted to evaluate the effectiveness of the in vivo treatment with PT on the premise that the G proteins that were ADP-ribosylated in vivo by PT treatment would not be subjected to ADP ribosylation in vitro. The results indicated in Figure 2 show PT-catalyzed ADP ribosylation of G\textsubscript{i}/G\textsubscript{o} proteins in hearts from 6- and 8-week-old control untreated and PT-treated SHR and WKY rats. As reported earlier, PT in the presence of [\alpha-\textsuperscript{32}P]NAD\textsuperscript{+} catalyzed the ADP ribosylation of a protein band of 40/41-kDa referred to as G\textsubscript{i}/G\textsubscript{o} in heart membranes solubilized by Lubrol PX at a final concentration of 0.3% from control untreated and PT-treated 6- and 8-week-old SHR and WKY rats (Figure 2A); however, it was significantly enhanced by ~45% in 6-week-old SHR compared with age-matched WKY rats, as determined by densitometric scanning (Figure 2B). On the other hand, the extent of ADP ribosylation of G\textsubscript{i}/G\textsubscript{o} was significantly lower in 6-week-old PT-treated SHR and WKY rats compared with untreated SHR and WKY rats, respectively. However, the extent of ADP ribosylation of G\textsubscript{i}/G\textsubscript{o} in 8-week-old PT-treated SHR was similar to that in untreated 8-week-old SHR, suggesting that the effect of PT had worn off at 8 weeks (6 weeks after the treatment).

**Effect of PT Treatment on G-Protein Levels**

Figure 3 shows the effect of PT treatment on the levels of G\textsubscript{i-2} (A) and G\textsubscript{i-3} (B) proteins in hearts from control untreated and 6- and 8-week-old PT-treated SHR and WKY rats, as determined by immunoblotting using specific antibodies: AS/7 against G\textsubscript{i-1} and G\textsubscript{i-2} and EC/2 against G\textsubscript{i-3}. AS/7 antibodies, which react with both G\textsubscript{i-1} and G\textsubscript{i-2}, recognized a single protein of 40 kDa, referred to as G\textsubscript{i-2} (G\textsubscript{i-1} has been shown to be absent from heart), on immunoblots of heart membranes from 6- and 8-week-old SHR and WKY rats. However, as reported earlier, the relative amount of immunodetectable G\textsubscript{i-2} was significantly enhanced by ~40% in SHR compared with WKY rats, as determined by densitometric scanning. On the other hand, the levels of G\textsubscript{i-2} were significantly reduced in 6-week-old PT-treated SHR and WKY rats compared with untreated SHR and WKY rats, whereas the levels of G\textsubscript{i-2} were similar in 8-week-old PT-treated and untreated SHR. Similarly, the EC/2 antibody recognized a single protein of 41 kDa referred to as G\textsubscript{i-3} in hearts from 6- and 8-week-old SHR and WKY rats (Figure 3A); however, it was significantly enhanced by ~40% in 6-week-old SHR compared with age-matched WKY rats, as determined by densitometric scanning (Figure 3B).
rats (Figure 3B); however, the relative amount of immuno-detectable $G_{i-3}$ was significantly augmented by $\sim20\%$ to $25\%$ in SHR compared with WKY rats, as determined by densitometric scanning. On the other hand, the levels of $G_{i-3}$ were significantly decreased in 6-week-old PT-treated SHR and WKY rats compared with control untreated SHR and WKY rats, respectively, whereas the levels of $G_{i-3}$ were similar in 8-week-old PT-treated and untreated SHR.

**Effect of PT Treatment on $G_i$ Functions**

To investigate whether the inactivation of $G_i$ proteins by PT-catalyzed ADP ribosylation was reflected in $G_i$ functions, the receptor-independent and -dependent $G_i$ functions were determined in hearts from 6- and 8-week-old SHR and WKY rats without and with PT treatment. Figure 4 shows the effect of varying concentrations of GTP$\gamma$S on forskolin (FSK)-stimulated adenylyl cyclase activity (receptor-independent $G_i$ functions) in 6- and 8-week-old control and PT-treated SHR and WKY rats. GTP$\gamma$S inhibited FSK-stimulated adenylyl cyclase activity in a concentration-dependent manner in hearts (Figure 4A) and mesenteric arteries (Figure 4B) from 6-week-old SHR and WKY rats; however, the extent of inhibition was significantly increased in SHR compared with WKY rats ($\sim20\%$ in WKY rats and $30\%$ in SHR). On the other hand, PT treatment reduced the extent of inhibition of FSK-stimulated adenylyl cyclase by GTP$\gamma$S in SHR and almost completely attenuated the effect in WKY rats.
Figure 4C shows the effect of GTPγS on FSK-stimulated adenylyl cyclase activity in hearts from 8-week-old control and PT-treated SHR and WKY rats. Adenylyl cyclase activity was determined in heart particulate fraction (A) and mesenteric arteries (B) from 6-week-old control and PT-treated SHR and WKY rats and heart particulate fraction (C) from 8-week-old control and PT-treated SHR and WKY rats in the absence or presence of 100 μmol/L FSK alone or in combination with various concentrations of GTPγS. Values are mean ± SEM of 3 separate experiments. Enzyme activities in the absence or presence of FSK in heart from control and PT-treated 6-week-old WKY rats and SHR were as follows, in pmol · (mg protein · 10 min)⁻¹: for WKY rats, 65.8 ± 2.6 (basal) and 2933.7 ± 49.4 (FSK); for PT-treated WKY rats, 71.2 ± 4.5 (basal) and 3645.9 ± 89.0 (FSK); for SHR, 71.9 ± 5.6 (basal) and 2119.7 ± 75.4 (FSK); and for PT-treated SHR, 66.1 ± 2.4 (basal) and 2854.5 ± 99.8 (FSK). Enzyme activities in the absence or presence of FSK in mesenteric arteries from control and PT-treated 6-week-old WKY rats and SHR were as follows, in pmol cAMP · (mg protein · 10 min)⁻¹: for WKY rats, 148.0 ± 5.1 (basal) and 5242 ± 38 (FSK); for PT-treated WKY rats, 135.6 ± 4.3 (basal) and 6655.7 ± 116 (FSK); for SHR, 169.2 ± 20.8 (basal) and 4453.8 ± 68.4 (FSK); and for PT-treated SHR, 188.4 ± 9.8 (basal) and 6182.4 ± 172.9 (FSK). Enzyme activities in the absence or presence of FSK in hearts from control and PT-treated 8-week-old WKY rats and SHR were as follows, in pmol cAMP · (mg protein · 10 min)⁻¹: for WKY rats, 93.4 ± 3.1 (basal) and 3047.6 ± 18.4 (FSK); and for PT-treated WKY rats, 232.8 ± 10.4 (basal) and 2905.5 ± 54.4 (FSK). *P < 0.05 for WKY rats vs SHR; †P < 0.05 for SHR vs PT-treated SHR.

Figure 5A shows the relationship between PT-induced inactivation of Gα proteins, 27,28 inhibited adenylyl cyclase activity in hearts from 6-week-old SHR and WKY rats; however, as reported earlier, 15,23 the extent of inhibition was significantly greater in SHR. For example, Ang II, C-ANP4-23, and oxotremorine inhibited adenylyl cyclase activity by ~15%, 20%, and 30%, respectively, in WKY rats and ~25%, 30%, and 45%, respectively, in SHR. PT treatment, on the other hand, almost completely attenuated Ang II-mediated, C-ANP4-23-mediated, and oxotremorine-mediated inhibition of adenylyl cyclase in WKY rats, whereas the percent inhibition of adenylyl cyclase by these hormones was significantly decreased in PT-treated SHR compared with untreated SHR.
In addition, Ang II, C-ANP 4-23, and oxotremorine inhibited adenylyl cyclase activity to the same degree in untreated and PT-treated SHR at 8 weeks compared with WKY rats (Figure 5B).

Effect of PT Treatment on Gs-Mediated Hormonal Stimulations of Adenylyl Cyclase Activity

Figure 6 shows the effects of inactivation of Gi proteins by PT treatment on the stimulatory effects of some agonists on adenylyl cyclase activity in 6- and 8-week-old SHR. Glucagon, isoproterenol, and 5'-N-ethylcarboxamidoadenosine stimulated adenylyl cyclase activity in hearts from 6- and 8-week-old SHR and WKY rats; however, as reported earlier,15,23 the augmentation of stimulation was significantly decreased in SHR compared with age-matched WKY rats. PT treatment, on the other hand, restored the decreased stimulations by 80% to 85% toward control WKY levels in 6-week-old SHR (Figure 6A) but not in 8-week-old SHR (Figure 6B).

Similarly, NaF- and FSK-mediated stimulations of adenylyl cyclase that were decreased in 6- and 8-week-old SHR compared with age-matched WKY rats were significantly reversed toward control WKY levels by 30% to 40% in 6-week-old PT-treated SHR but not in 8-week-old PT-treated SHR (Figure 7).

Discussion

We and others have previously shown an increased expression of Gi proteins in hearts and aortas from SHR, whereas the levels of Go and Gs were not altered.15 The enhanced expression of Gi proteins occurs before the onset of hypertension and suggests that enhanced expression of Gi proteins and resultant decreased levels of cAMP in response to various hormones, including Ang II, may be one of the contributing factors in the pathogenesis of hypertension.23

In the present studies, we report that the inactivation of Gi proteins by a single intraperitoneal injection of PT in 2-week-old prehypertensive SHR attenuates the development of high BP up to 6 weeks of age, after which BP starts increasing and reaches the same level as found in SHR at 8 weeks, which may be due to the possibility that PT is no longer effective or may be eliminated from the system. This notion is further supported by the fact that a second injection of PT at 6 weeks delays the increase in BP for another week, whereas a second injection at 8 weeks decreases the BP significantly but not to the control WKY level. On the other hand, no significant reduction in BP was evoked by PT in WKY rats. The reduction in BP by PT treatment in 12-week-old SHR has been reported.29 In addition, Kost et al30 have also shown that PT treatment reduced BP in adult SHR (4 to 7 months old).
with established hypertension but not in WKY rats; however, this is the first study showing the attenuation of the development of high BP in SHR by PT treatment.

PT has been reported to ADP-ribosylate and inactivate $G_i$ as well as $G_o$ proteins; these occurrences result in the inhibition of a variety of $G_i$-mediated hormonal and peptidergic effects. The inactivation of $G_i$ protein attenuates the GTP-dependent and hormone receptor–mediated inhibition of adenylyl cyclase and results in the augmentation of cAMP levels.

In the present study, we have shown that intraperitoneal injection of PT into 2-week-old prehypertensive SHR and their age-matched WKY rats results in similar in vivo ADP ribosylation of $G_i$ proteins as well as in the decreased levels of active unribosylated $G_i$-2 and $G_i$-3 proteins in 6-week-old rats. These data suggested that the distribution of toxin was not different in the two strains. However, the attenuation of BP by PT treatment was observed only in SHR and not in WKY rats, suggesting the implication of enhanced expression of $G_i$ protein in the development of high BP in SHR. Our results are in accordance with the studies of other investigators, who also did not observe any effect of PT on BP in WKY rats. However, $G_o$ proteins that are also subjected to ADP ribosylation by PT may not be implicated in the reduction of BP, because their levels were shown not to be altered in SHR. Our results are consistent with studies reported earlier showing that intraarterial injection of PT into rats decreased the level of $G_i$ proteins in the pancreas after 24 and 48 hours of PT treatment and that this decrease was accompanied by the appearance of a band that was an inactive ADP-ribosylated form of $G_i$-2 with less mobility ($\approx 2$-kDa shift in molecular weight). However, we were unable to detect the inactivated ADP-ribosylated form of $G_i$-2 or $G_i$-3 in the present study, which may be due to the possibility that more extended treatment of rats with PT (4

**Figure 6.** Effect of PT-induced inactivation of $G_i$ proteins on hormonal stimulation of adenylyl cyclase activity. Adenylyl cyclase activity was determined in the presence of adenosine deaminase and 10 $\mu$mol/L GTP alone (basal) or in combination with 1 $\mu$mol/L glucagon, 50 $\mu$mol/L isoproterenol, and 10 $\mu$mol/L 5'-N-ethylcarboxamidoadenosine (NECA) in heart particulate fractions from 6-week-old (A) and 8-week-old (B) SHR and WKY rats. Basal enzyme activities in 6-week-old WKY rats, SHR, PT-treated WKY rats, and PT-treated SHR were 45.1 ± 3.5, 49.8 ± 1.2, 49.7 ± 1.3, and 46.6 ± 4.6 pmol cAMP · (mg protein · 10 min)$^{-1}$, respectively, and in 8-week-old WKY rats, SHR, PT-treated WKY rats, and PT-treated SHR, these values were 168.7 ± 15.1, 175.8 ± 16.0, 309.6 ± 5.8, and 302.9 ± 8.6 pmol cAMP · (mg protein · 10 min)$^{-1}$, respectively. Values are mean ± SEM of 3 separate experiments. NS indicates not significant. *$P<0.05$; **$P<0.01$; and ***$P<0.001$. 

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Li and Anand-Srivastava  PT Attenuates Development of High BP in SHR 7
weeks) may result in the degradation of the ADP-ribosylated form of Gi proteins. The fact that PT treatment results in the inactivation of Gi proteins and in decreased levels of active Gi proteins in the hearts of 6-week-old SHR compared with untreated SHR has been demonstrated by decreased inhibition of FSK-stimulated adenylyl cyclase activity by GTPgS as well as by decreased inhibition of adenylyl cyclase by Ang II, C-ANP 4-23, and oxotremorine. Our results are in agreement with previous studies showing the attenuation of enhanced renal vascular responsiveness to Ang II in SHR by PT treatment. In addition, PT-induced inactivation of Gi proteins also restores the diminished stimulation of adenylyl cyclase by stimulatory hormones and results in increased levels of cAMP. However, the increased levels of cAMP in the heart may not be responsible for the attenuation of the development of high BP but may contribute to the restoration of impaired cardiac functions in hypertension.

The inactivation of Gi proteins by PT injection was not only confined to the heart; the Gi proteins of mesenteric artery resistance vessels, which are intimately involved in the regulation of BP, were also inactivated by intraperitoneal injection of PT, as shown by decreased inhibition of FSK-stimulated adenylyl cyclase by GTPgS. Furthermore, Komatsu et al have also shown ADP-ribosylation and inactivation of Gi-2 protein in the pancreas by intraperitoneal injection of PT. In addition, PT-induced inactivation of Gi proteins has also been shown to affect renal functions in SHR, as has been indicated by increased renal blood flow and decreased renal vascular resistance in SHR; these findings suggest that a Gi-mediated pathway may contribute to increased vascular tone in the SHR kidney. The role of cAMP in diuresis and natriuresis has been demonstrated. Proximal tubules from SHR have been shown to generate decreased levels of cAMP in response to dopamine and Ang II, which may contribute to decreased diuresis and increased sodium retention in the SHR. Taken together, it may be possible that intraperitoneal injection of PT, which inactivates Gi proteins and therefore increases the formation of cAMP to hormonal stimuli in the proximal tubule, results in increased diuresis and decreased sodium retention and may thus contribute to the attenuation of the development of high BP at 6 weeks in SHR. However, Kost et al did not observe any significant increase in urine volume and sodium excretion after intravenous injection of PT into adult SHR. The reason for lack of a PT effect on natriuresis and diuresis is not clear in their studies and may be due to the possibility that the duration of PT treatment was too short (3 to 5 days) to observe these effects. Thus, it may be suggested that the inactivation of Gi proteins in various target tissues (including mesenteric artery resistance vessels, renal vasculature, and proximal tubules) by intraperitoneal injection of PT and the resultant increased levels of cAMP may contribute (by decreasing vascular resistance and salt and water retention and increasing vascular permeability) to the attenuation of development of high BP in SHR up to 6 weeks.

Figure 7. Effect of PT-induced inactivation of Gi proteins on NaF- and FSK-stimulated adenylyl cyclase activity. Adenylyl cyclase activity was determined in the presence of adenosine deaminase alone (basal) or in combination with 50 μmol/L FSK or 10 mmol/L NaF in heart particulate fractions from 6-week-old (A) and 8-week-old (B) SHR and WKY rats, as described in Materials and Methods. GTP or GTPgS was omitted from the reaction mixture. Basal enzyme activities in 6-week-old WKY rats, SHR, PT-treated WKY rats, and PT-treated SHR were 26.3 ± 1.9, 16.7 ± 0.5, 24.5 ± 1.2, and 23.3 ± 1.2 pmol cAMP (mg protein · 10 min)-1, respectively, and in 8-week-old WKY rats, SHR, PT-treated WKY rats, and PT-treated SHR, these values were 104.7 ± 11.5, 109.8 ± 6.9, 143.6 ± 3.2, and 138.9 ± 9.4 pmol cAMP (mg protein · 10 min)-1, respectively. Values are means ± SEM of 3 separate experiments. NS indicates not significant. **P < 0.01; ***P < 0.001.
On the other hand, the levels of $G_{i,-2}$ and $G_{i,-3}$ in PT-treated 8-week-old SHR that were increased to the same extent as levels found in untreated SHR may be due to the possibility that PT at 8 weeks was ineffective to ADP-ribosylate and thereby decrease the enhanced levels of $G_i$ proteins. The enhanced expressions/functions of $G_i$ proteins in the heart (leading to decreased levels of cAMP) may not contribute to the pathogenesis of hypertension but may be implicated in decreased cardiac function (contractility) in hypertension, which may lead to the development of heart failure. However, the increased levels of $G_{i,-2}$ and $G_{i,-3}$ and the resultant decreased levels of cAMP in various target tissues in PT-treated SHR at 8 weeks may be responsible for impaired cellular functions, including increased vascular resistance, increased salt and water retention, and decreased capillary permeability, all of which may contribute to the augmentation of BP.

In conclusion, we have provided the first evidence showing that inactivation of $G_i$ proteins by PT in prehypertensive SHR attenuates the development of high BP. From these studies, it can be suggested that the novel strategies that target $G_i$ proteins could be designed to treat hypertension.

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References


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