Aging and prostacyclin responses in aorta and platelets from WKY and SHR rats

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Gomez E, Schwendemann C, Roger S, Simonet S, Paysant J, Courchay C, Verbeuren TJ, Féletou M. Aging and prostacyclin responses in aorta and platelets from WKY and SHR rats. Am J Physiol Heart Circ Physiol 295: H2198–H2211, 2008. First published September 26, 2008; doi:10.1152/ajpheart.00507.2008.—In spontaneously hypertensive rat (SHR) aorta, prostacyclin is an endothelium-derived contracting factor contributing to the endothelial dysfunction. This study was designed to determine whether the impairment of the prostacyclin response is influenced by aging and whether such a dysfunction is observed in platelets. Isometric tension was measured in aortic rings, and aggregation was studied in platelet-rich plasma taken from 3-, 6-, and 15-mo-old Wistar-Kyoto rats (WKY) and SHR. In aorta from 3- and 6-mo-old WKY, prostacyclin and beraprost [prostacyclin receptor (IP) agonists] produced relaxations that were enhanced by Triplion (thromboxane-prostanoid receptor antagonist). In 15-mo-old WKY, the relaxations to beraprost were maintained, but not those to prostacyclin. In SHR aorta, prostacyclin or beraprost produced no or minor relaxations, which, in younger SHR, were enhanced by Triplion. In both strains, the relaxations were inhibited by CAY-10441 (IP receptor antagonist). The relaxations to forskolin and isoproterenol were reduced with aging. When compared with those of WKY, the relaxations to isoproterenol were reduced in 3- but not in 6- or 15-mo-old SHR, whereas those to forskolin were consistently diminished at any given age. Whatever the age, prostacyclin and beraprost produced CAY-10441-sensitive inhibitions of ADP-induced platelet aggregation. Both agonists were more potent in SHR than in WKY. Therefore, in platelets from WKY and SHR, the IP receptor-dependent antiaggregant response is functional and maintained during aging. In aorta from WKY those responses are reduced by aging and, in SHR, are already compromised at 3 mo. This dysfunction of the IP receptor is only partially explained by a general dysfunction of the adenylyl cyclase pathway.

smooth muscle; prostacyclin receptor; endothelium-derived contracting factors; endothelial dysfunction; spontaneously hypertensive rats; Wistar-Kyoto rats

ENDOTHELIAL DYSFUNCTION is a generic term that encompasses many different disorders (15). In the genetic model of spontaneously hypertensive rats (SHR), the endothelial dysfunction is attributed to the release of endothelium-derived contracting factors (EDCF) that counterbalances the effect of nitric oxide (NO) with no or minor alteration in the production of the latter (35). In response to acetylcholine, the endothelium-dependent contraction involves the production of reactive oxygen species, the activation of cyclooxygenase-1, the diffusion of EDCF, and the subsequent stimulation of thromboxane-prostanoid (TP) receptors on vascular smooth muscle. Since inhibitors of thromboxane synthase do not affect the endothelium-dependent contraction to acetylcholine, thromboxane A2 is not the EDCF released by the muscarinic agonist (20, 35, 62, 63), although it contributes to the endothelium-dependent contractions elicited by other stimuli such as ATP, endothelin, and the calcium ionophore A-23187 (20, 22, 55). As a matter of fact, in the aorta of SHR the EDCFs released by acetylcholine have been identified as PGH2 and paradoxically prostacyclin (19, 20, 48). In the aorta of SHR and Wistar-Kyoto rats (WKY), prostacyclin is the principal metabolite of arachidonic acid released by acetylcholine, the endothelial cells being the predominant site of its synthesis (20, 21, 39, 40). Prostacyclin is generally described as an endothelium-derived vasodilator, which, by stimulating its G protein-coupled receptor [prostacyclin receptors (IP)], produces smooth muscle relaxation (59). However, in aorta from mature SHR, prostacyclin and its stable analog iloprost do not produce relaxations (20, 31, 48). In contrast, prostacyclin activates the smooth muscle thromboxane A2/endoperoxide TP receptor and produces contraction (20, 31, 48). Endothelium-dependent contractions are exacerbated during the aging process in the SHR and are also generated in arteries of aging normotensive animals (17, 25, 29, 38).

Prostacyclin is not only a vasodilator substance but also a potent antithrombotic and antiplatelet agent, exerting most of the latter effects via the activation of platelet IP receptors (54). Although several types of G proteins are likely to be coupled to the IP receptor, in platelets and vascular smooth muscle cells, the G alpha-adenylate cyclase-cAMP-PKA pathway is thought to be the preponderant signaling system responsible for preventing platelet activation and producing vascular relaxation (54). High blood pressure causes functional changes in both vascular endothelial cells and platelets, and, in humans, hypertension is associated with increased aggregant responses (43). In SHR, in different in vivo pathological models, an increase in thrombogenicity has been reported (34, 41), whereas in platelets, in vitro, alterations in calcium handling and in adenylyl cyclase activation as well as abnormal platelet aggregation have been observed (3, 46).

The purpose of the present work was to determine in isolated aorta and platelets from young (3-mo-old, when the hypertension is just established), mature (6-mo-old, when the hypertension is stabilized), and in aging (15-mo-old) WKY and SHR whether the dysfunction associated with the activation of the IP receptor in aortic smooth muscle cells is influenced by aging and whether this dysfunction is also observed in platelets.

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MATERIALS AND METHODS

This study was performed in agreement with the National Research Council Guide for the Care and Use of Laboratory Animals and was approved by the ethical committee of the Institut de Recherches Servier.

Male SHR and WKY (3-, 6-, and 15-mo-old; Charles River, l’Arbresle, France) were anesthetized with pentobarbital sodium (50 mg/kg ip), and the arterial blood pressure was measured from the carotid artery.

In some rats, the aorta was then dissected free, excised, and placed in cold modified Krebs-Ringer bicarbonate solution of the following composition (in mmol/l): 118 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 KH2SO4, 25.0 NaHCO3, and 0.026 edetate calcium disodium; and 11.1 glucose (control solution). The endothelium could be removed from segments of various lengths by infusing a saponin solution (1 mg/ml, for 20 s) that was subsequently flushed with control solution (14). The aorta was then cut into rings (4 to 5 mm in length).

In other rats, the second carotid artery was also catheterized and blood was drawn with a syringe and collected on sodium citrate (0.109 M, 1 volume of citrate for 9 volumes of blood). The whole blood was centrifuged at room temperature (1,000 rpm, 170 g, for 20 min), and the platelet-rich plasma (PRP) was collected and then the platelet-poor plasma (PPP) was obtained after a second centrifugation (4,000 rpm, 3,000 g, for 10 min). Platelet count was performed in the whole blood, PRP, and PPP (Beckman Coulter). The PRP count was adjusted to 800,000 platelets/l by adding the appropriate volume of PPP.

Isometric tension recording. The rings were suspended in organ chambers (20 ml), which contained control solution (37°C) aerated with 95% O2-5% CO2. They were connected to a force transducer to record isometric contraction. They were stretched progressively to reach the optimal point of their length-active tension relationship (1101 g). Drug incubation time was at least 30 min. In most of the studies, aortic rings were contracted with the $\alpha_1$-adrenergic agonist phenylephrine. In experiments involving the relaxing effect of isoproterenol,

![Prostacyclin (WKY) Diagram]

**Fig. 1.** Prostacyclin (10$^{-9}$ to 10$^{-5}$ M)-induced relaxation in isolated aortic rings with and without endothelium of 3-, 6-, and 15-mo-old Wistar-Kyoto rats (WKY): effect of Triplion (100 nM). Aortic rings were contracted with phenylephrine. Data are shown as means ± SE ($n = 6$). *Statistically significant effect caused by the presence of the thromboxane-prostanoid (TP) receptor antagonist Triplion (100 nM).

### Table 1. Characteristics of 3-, 6-, and 15-mo-old WKY and SHR

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<th>WKY</th>
<th>SHR</th>
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<tr>
<td></td>
<td>3 mo</td>
<td>6 mo</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>297±3</td>
<td>327±9*</td>
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<tr>
<td>Systolic arterial blood pressure, mmHg</td>
<td>139±3</td>
<td>145±3</td>
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<tr>
<td>Platelet count in PRP, $\times$ 1,000/μl</td>
<td>890±45</td>
<td>986±25</td>
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<td>n</td>
<td>32</td>
<td>38</td>
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Data are means ± SE; $n$, number of different rats. WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rat; PRP, platelet-rich plasma. *Statistically significant difference between 3-mo-old and older rats within the same strain; †statistically significant difference between 6-mo-old and the older rats within the same strain; ‡statistically significant difference between WKY and SHR rats at a given age (ANOVA followed by a Bonferroni’s multiple comparison test).
aortic rings were contracted with the TP receptor agonist U-46619, since the α1-adrenergic blocker prazosin was used to prevent the secondary contractions produced by isoproterenol at concentrations higher than 1 μM. In either case the contractions elicited represented ~75% of the reference contraction to KCl (60 mM), which was performed in each ring before the beginning of the experimental protocol. Concentration-response curves of relaxing agonists were obtained in a cumulative manner. Each ring was exposed to only one set of cumulative concentrations of a given agonist. Relaxing responses were expressed as a percentage of the maximal relaxation produced by papaverine (0.1 mM) obtained in each ring at the end of the experiment.

Platelet aggregation. Platelet aggregation was performed with an optical aggregometer (Chrono-log; Kordia Life Sciences, Leiden, The Netherlands) at 37°C with 250 μl of PRP placed in glass cuvette containing a disposable stir bar for constant stirring (1,000 rpm). Before stirring, drugs or solvents were incubated for 5 min before the addition of prostacyclin (2 min incubation), beraprost, or forskolin (5 min incubation). Platelet aggregation was induced by the subsequent addition of ADP and followed for 8 min. The maximal aggregation (percentage) and the area-under-the-curve parameter were calculated using the Aggrolink software (Chrono-log); autologous PPP provided a signal representing 0% aggregation. To take into account reversible aggregation, the parameter selected to analyze the data obtained in the various protocols was the area under the curve.

Drugs. Forskolin, isoproterenol, papaverine, phenylephrine, prazosin, saponin, and SQ-22536 were obtained from Sigma (La Verpillère, France). Beraprost, prostacyclin (PGI2), U-46619 (9,11-dideoxy-9α,11α-epoxymethano prostaglandin F 2α), and CAY-10441 (4,5-dihydro-N-[4-[4-(1-methylethoxy)phenyl][methyl]phenyl]-1H-imidazol-2-amine) were purchased from Cayman Chemical (Ann Arbor, MI). Triplion [S-18886 or terutroban (3-[[(6-amino-(4-chlorobenzensulfonyl)-2-methyl-5,6,7,8-tetrahydronaphthalen-1-yl)propionic acid]) was synthesized at the Institut de Recherches Servier (Suresnes, France). Drug concentrations are expressed as final molar concentrations in the organ bath solution and aggregation cuvette.

Data analysis. Data are expressed as means ± SE; n refers to the number of rats from which the tissues were taken. The IC50 (concentration of agonist causing a relaxation representing 50% of the reference relaxation to 100 μM papaverine) was calculated using the Michaelis-Menten equation and nonlinear regression that included all the data points. Statistical analysis was performed by two-tailed Student’s t-test for control and treatment comparisons and by ANOVA1 or ANOVA2 analysis for multiple comparisons followed by a Bonferroni post hoc test, where appropriate. Differences were considered to be statistically significant when P < 0.05.

RESULTS
Characteristics of the two strains. The weight of male WKY significantly increased during aging (from 3 to 15 mo) without any significant changes in systolic arterial blood pressure and number of circulating platelets. In SHR, the body weight also increased during this time frame. The systolic arterial blood pressure increased from 3 to 6 mo of age and then leveled off. Platelet count was similar in 3- and 6-mo-old SHR and then slightly decreased. At any given age, compared with WKY, SHR rats have a reduced body weight, a significantly higher arterial blood pressure. The number of circulating platelets is also significantly higher in SHR than in WKY at 3 and 6 mo of age (Table 1).

Aorta. In isolated aortic rings of 3- and 6-mo-old WKY contracted with phenylephrine, prostacyclin (10^-9 to 10^-5 M)
produced similar and biphasic concentration-dependent relaxations that were at the two ages significantly smaller in rings without endothelium than in rings with endothelium. Beraprost (10^{-9} to 10^{-5} M) produced biphasic relaxations in both rings with and without endothelium. In all groups, Triplion (100 nM) significantly enhanced the relaxations and suppressed the differences associated with endothelial denudation. In 15-mo-old WKY, the relaxation to prostacyclin was virtually abolished, but that to beraprost was preserved. The presence of Triplion unmasked a small relaxation to prostacyclin and potentiated that to beraprost (Figs. 1 and 2). In aortic rings of SHR, prostacyclin produced no or minor relaxations and in that of younger animals (3 and 6 mo old) evoked contractions at concentrations higher than 10^{-6} M. Triplion inhibited the secondary contractions and in some instances restored small relaxations. In younger animals, beraprost produced biphasic relaxations but only small contractions in 15-mo-old SHR. In the presence of Triplion, the secondary contractions were inhibited and a relaxing effect of beraprost could be observed in the aorta of the 3- and 6-mo-old rats (Figs. 3 and 4). The relaxations to prostacyclin and beraprost were significantly impaired in the aortic rings of SHR compared with those of WKY, whatever the age or the experimental conditions (in rings with or without endothelium and in the absence or presence of Triplion), with one exception: the relaxations to prostacyclin in the 15-mo-old animals, which were virtually abolished in both strains (Figs. 1–4).

In aortic rings with endothelium of 3- and 6-mo-old WKY, the relaxations to prostacyclin and beraprost were abolished by the presence of the IP receptor blocker CAY-10441 (100 nM; Fig. 5). In aortic rings of SHR, this antagonist also prevented the small relaxations produced by beraprost, but the secondary contractions evoked by either prostacyclin or beraprost were not affected (Fig. 6). In rings without endothelium from both strains and in the presence of Triplion, CAY-10441 significantly shifted to the right the concentration-response curves to either prostacyclin or beraprost (Figs. 5 and 6). In 15-mo-old WKY aortic rings, the relaxations to beraprost were reversed by the addition of CAY-10441 (1 μM; Table 2).

In rings contracted with U-46619 (in the presence of the α1-adrenergic blocker prazosin; 100 nM), isoproterenol (10^{-9} to 10^{-5} M) produced concentration-dependent relaxations. At 3 mo of age the relaxations were significantly larger in aortic rings with or without endothelium from WKY than in those from SHR. In aortic rings from 6-mo-old WKY, the relaxation to the β-adrenergic agonist was impaired compared with that observed in younger rats (significantly in rings with endothelium). In contrast, in SHR, the relaxations to isoproterenol were not significantly affected by aging and at 6 mo of age were no longer significantly different from those observed in aortic rings of the control WKY. In 15-mo-old rats, the relaxations to isoproterenol were further diminished but remained similar in both strains (Fig. 7).

In aortic rings without endothelium, the adenylate cyclase activator forskolin (10^{-9} to 10^{-6} M) produces complete relaxations in both strains at any given age. The effect of forskolin was affected by aging (IC_{50} in WKY, 26, 45, and 170 nM at 3, 6, and 15 mo of age, respectively; and in SHR, 96, 148, and
576 nM at 3, 6, and 15 mo of age, respectively). The concentration-response curves to forskolin were consistently shifted to the right in SHR compared with that of WKY, by approximately a factor 3 (statistically significant at 3 and 15 mo; ANOVA; $P < 0.05$; Fig. 8).

**Platelets.** In both 3- and 6-mo-old WKY and SHR, ADP ($10^{-7}$ to $3 \times 10^{-5}$ M) produced a concentration-dependent platelet aggregation. The maximal aggregations were not significantly different in WKY versus SHR, but ADP was significantly more efficient in the WKY than in the SHR. ADP at the concentration of $10^{-8}$ M in the 3-mo-old rat and $30 \times 10^{-6}$ M in 6- and 15-mo-old rats produced maximal platelet aggregations (Fig. 9).

Prostacyclin ($10^{-8}$ to $10^{-6}$ M) and beraprost ($10^{-8}$ to $10^{-6}$ M) produced concentration-dependent inhibition of ADP-induced platelet aggregation in WKY and SHR, and their effects were not affected or minimally enhanced by aging. However, at any given age, both prostacyclin and beraprost were significantly more potent in preventing ADP-induced platelet aggregation than in the WKY. ADP at the concentration of $10 \mu$M in the 3-mo-old rat and $30 \mu$M in 6- and 15-mo-old rats produced maximal platelet aggregations (Fig. 9).

Prostacyclin ($10^{-8}$ to $10^{-6}$ M) and beraprost ($10^{-8}$ to $10^{-6}$ M) produced concentration-dependent inhibition of ADP-induced platelet aggregation in WKY and SHR, and their effects were not affected or minimally enhanced by aging. However, at any given age, both prostacyclin and beraprost were significantly more potent in preventing ADP-induced platelet aggregation in SHR than in WKY (Figs. 10 and 11). Triplion (0.1 $\mu$M) did not affect the inhibitory effect produced by prostacyclin (data not shown). In both WKY and SHR platelets, the presence of CAY-10441 (0.1 or 1 $\mu$M) reversed the inhibitory effect produced by either prostacyclin or beraprost (Figs. 12 and 13). SQ-22536 (200 $\mu$M), an inhibitor of adenylate cyclase, partially but significantly reversed the inhibitory effect of either prostacyclin or beraprost (data not shown).

Forskolin ($10^{-7}$ to $3 \times 10^{-6}$ M) also produced a concentration-dependent inhibition of ADP-induced platelet aggregation. However, the effect of the adenylate cyclase activator was significantly attenuated in platelets from 15-mo-old rats compared with the effects observed either at 3 or 6 mo of age. Again, and at any given age, forskolin was more potent in SHR than in WKY (Fig. 14).

In both strains, Triplion (100 nM), CAY-10441 (1 $\mu$M), and SQ-22536 (200 $\mu$M) did not affect ADP-induced platelet aggregation (data not shown).

**DISCUSSION**

The present study shows that in SHR the functionality of the IP receptor is markedly altered in vascular smooth muscle cells as early as 3 mo of age but is completely preserved in platelets. Furthermore, although a general dysfunction of the signaling pathways involving the stimulation of adenylate cyclase is observed in SHR and with aging in both WKY and SHR, in SHR the dysfunction associated with the IP receptor is more severe than the dysfunction associated with $\beta$-adrenoceptor stimulation or direct activation of the adenylate cyclase.

In aortic rings of WKY, prostacyclin and beraprost, a synthetic prostacyclin analog agonist of the IP receptor (1, 52), produced a biphasic response and a relaxation followed by a contraction. The aging process affected the prostacyclin-induced and, to a lesser extent, the beraprost-induced relaxations at the latest time point. The responses to prostacyclin are significantly reduced in aortic rings of WKY without endothelium compared with those with endothelium. This could be attributed to the release of an endothelium-derived relaxing.
factor as it has been previously demonstrated in other arteries. The IP receptor can be expressed in endothelial cells (30), and its activation can be associated with NO release (51). However, whether a functional IP receptor is expressed in the rat aortic endothelial cells remains uncertain. The potentiating effect of the endothelial cells on IP receptor-dependent relaxations can also be explained by the activation of endothelial NO synthase, which occurs during isometric contractions (16). This basal release of NO could inhibit the effect of TP receptor activation. Indeed, in quiescent rat aorta, prostacyclin produces contraction by activating TP receptors (20), and in the present study, in phenylephrine-contracted aortic rings, the relaxing effects of prostacyclin were biphasic, fading away at elevated concentrations. The presence of the specific TP receptor antagonist Triplion (53) enhanced the relaxations in aortic rings with and without endothelium but much more so in vessels without endothelium. In the rat aorta, NO is a potent functional antagonist of TP receptor activation. It produces a major rightward shift in the concentration-response curves to full agonists, such as U-46619, and prevents the contraction in response to partial agonists, such as prostacyclin (20). Therefore, the simultaneous activation of TP receptors, even by a weak and partial agonist of this receptor such as prostacyclin, can markedly blunt vascular relaxation (20, 26).

The relaxations to either prostacyclin or beraprost were inhibited by CAY-10441, a specific IP receptor antagonist (8), confirming that the relaxing effects of these substances involved the stimulation of IP receptors. In the presence of the endothelium and without TP receptor blockade, the IP receptor antagonist CAY-10441 completely blocks the relaxation to either prostacyclin or beraprost. In the presence of Triplion, the amplitude of the relaxations was enhanced without any significant modification in the IC_{50}. Under these conditions, CAY-10441 produced a rightward shift of the concentration-response curves. The IP receptor antagonist appears more potent in inhibiting prostacyclin-induced relaxation than beraprost. This suggests that beraprost could elicit smooth muscle relaxations by activating other receptor(s) than the IP receptor, and indeed, nonselective effects of beraprost have previously been reported (26, 28). The lack of selectivity of beraprost could have explained the persistent relaxations to this agonist in 15-mo-old WKY, whereas that to prostacyclin had virtually disappeared. However, since CAY-10441 was able to reverse the relaxations produced by beraprost, this effect is dependent on IP receptor stimulation. In aortic rings from old WKY, the enhanced effect of beraprost compared with that of prostacyclin could be attributed to a higher intrinsic efficacy toward the IP receptor of the former compared with the latter.

In aortic rings of SHR, the relaxation to prostacyclin was virtually nonexistent but beraprost produced small relaxations in 3- and 6-mo-old rats. Both compounds produced secondary contractions that were inhibited by Triplion. In those younger rats and in the presence of the TP receptor antagonist, a quantifiable relaxation to prostacyclin was unveiled and the
relaxations to beraprost were enhanced. These relaxations also involved the activation of IP receptors since they were inhibited by CAY-10441. Nevertheless, the relaxations to prostacyclin and beraprost were significantly impaired in the aortic rings of SHR compared with those of WKY, whatever the age or the experimental conditions with the exception of the relaxations to prostacyclin in the 15-mo-old animals, which were virtually abolished in both strains.

In vascular smooth muscle cells, the preponderant signaling system associated with IP receptor stimulation is the Gs-adenylate cyclase pathway (54). Forskolin, a direct activator of adenylate cyclase, produced relaxations in isolated aortic rings from both strains. Although complete relaxations were observed, the responses were blunted by aging and were significantly impaired in SHR aorta compared with that of WKY at any given age.

The relaxations produced by isoproterenol, an agonist of \(\beta\)-adrenergic receptors, also involve preponderantly but not exclusively adenylate cyclase activation (37, 49, 50). In aortic rings of 3-mo-old SHR, the relaxations to isoproterenol were significantly reduced compared with those of WKY. These observations are in agreement with earlier work showing that vascular relaxations in response to \(\beta\)-adrenergic stimulation and, more generally in response to stimuli involving the stimulation of adenylate cyclase, are reduced in SHR (2, 9, 18, 36). The \(\beta\)-adrenergic dysfunction appears early, preceding the development of hypertension, and was attributed with either a decrease in G protein Gs (18, 36) or an increase in Gi function and/or expression (4).

In vascular smooth muscle cells, the preponderant signaling system associated with IP receptor stimulation is the Gs-adenylate cyclase pathway (54). Forskolin, a direct activator of adenylate cyclase, produced relaxations in isolated aortic rings from both strains. Although complete relaxations were observed, the responses were blunted by aging and were significantly impaired in SHR aorta compared with that of WKY at any given age.

Table 2. Effect of 1 \(\mu\)M CAY-10441 on 10 \(\mu\)M beraprost induced changes in tension in phenylephrine-contracted aortic rings from 15-mo-old WKY with or without the presence of 100 nM Triplion

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<th>Control</th>
<th>Triplion</th>
<th>Control</th>
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<tr>
<td>With Endothelium</td>
<td></td>
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<tr>
<td>Beraprost</td>
<td>104 ± 8</td>
<td>51 ± 20</td>
<td>88 ± 13</td>
<td>51 ± 13</td>
</tr>
<tr>
<td>+ CAY-10441</td>
<td>127 ± 3*</td>
<td>85 ± 19*</td>
<td>103 ± 2*</td>
<td>70 ± 16*</td>
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Data are means ± SE in percentage of changes in tension (n = 3). *Statistically significant difference induced by the addition of CAY-10441 (paired t-test; P < 0.05).
old WKY and further reduced in the 15-mo-old rats. These results confirm that responses to direct activators of adenylate cyclase, as those to phosphodiesterase inhibitors and cell-permeable cAMP analogs, are less affected by aging than \(\beta\)-adrenergic responses (12, 45), indicating that the effects of aging involve events upstream of adenylate cyclase activation. Interestingly, in the aorta of 6- and 15-mo-old SHR, the responses to isoproterenol are no longer different from those observed in WKY of the same age.

Taken together, these results indicate that, as early as 3 mo of age, in vascular smooth muscle cells of SHR the functionality of the IP receptor is markedly altered and, although an early general dysfunction of adenylate cyclase can be demonstrated in SHR, the impairment of the IP receptor-dependent responses is likely to involve additional mechanisms. The absence of IP receptor-dependent relaxation in SHR aorta could have been attributed to a reduced expression of this receptor. Indeed, Numaguchi et al. (42) have suggested in both old WKY and SHR.
WKY and SHR that the IP receptor mRNA expression decreases with age and that, at any given age, it is slightly but systematically less expressed in SHR than in WKY. However, a recent study has ruled out this hypothesis and showed that aging and hypertension does not significantly modify the genomic expression of the IP receptor in the aorta of these rat strains (57). Alternatively, prostacyclin and its analogs can produce vascular relaxations via cAMP-independent mechanisms, for instance by activating inward rectifier and large conductance calcium-activated potassium channels (47, 61). Whether these additional mechanisms contribute to IP receptor-dependent relaxation in rat aortic rings and whether they could be altered in SHR remain to be determined.

SHR at 3 and 6 mo of age have an increased number of circulating platelets, a phenomenon that has been associated with an increase in generation and not with an extended half-life of platelets (23). Under our experimental conditions, SHR platelets compared with those of WKY were hyporeactive in response to ADP, in agreement with earlier observations (33, 56). In sharp contrast with what is observed in vascular smooth muscle cells, the antiaggregant effects of prostacyclin, beraprost, and forskolin were fully preserved and even enhanced in platelets of SHR rats compared with those of WKY. Only forskolin showed an age-dependent reduction of its antiaggregant effect. Although experiments were tentatively performed with equieffective concentrations of the aggregating agent ADP, the hyporeactivity of SHR platelets may have favored the inhibitory effects of these compounds. Additionally, a decrease in Gi expression in SHR platelets can enhance the responsiveness to forskolin and to agonists activating receptors positively coupled to adenylate cyclase (3, 10). The inhibitory effects of prostacyclin and beraprost were reversed by the IP receptor antagonist CAY-10441, confirming the involvement of IP receptors (8, 27). In platelets, the IP receptor is predominantly coupled to the adenylate cyclase pathway (54) and cAMP-elevating agents are potent inhibitors of platelet activation (58). In both strains, forskolin was a potent inhibitor of platelet aggregation and SQ-22536, an adenylate cyclase inhibitor (6, 11, 24), partially reversed the effects of beraprost and prostacyclin. These results indicate that in platelets from SHR and WKY, the IP receptor is coupled to adenylate cyclase and is fully functional. Prostacyclin (or beraprost) can stimulate TP receptors in isolated aortic rings, but there is no evidence for this phenomenon in platelets. This observation can be explained by the fact that although the activation of TP receptors does produce platelet aggregation in PRP from WKY and SHR, elevated concentrations of potent TP receptor agonists, such as U-46619, are required (32). Prostacyclin, being a weak and partial agonist of the TP receptor, did not produce an aggregant response in the range of concentration tested.

In conclusion, in SHR the functionality of the IP receptor is markedly altered in aortic vascular smooth muscle cells as early as 3 mo of age but is completely preserved in platelets. Furthermore, this dysfunction appears more severe for the IP receptor and is only partially explained by a general dysfunc-

![Platelet Aggregation](image_url)

**Fig. 9.** Aggregation of platelets in platelet-rich plasma of 3-, 6-, and 15-mo-old WKY and SHR. **Top:** ADP (10^{-7} to 3 \times 10^{-5} M)-induced platelet aggregation shown as maximal aggregation (percentage). **Bottom:** ADP (10^{-7} to 3 \times 10^{-5} M)-induced platelet aggregation shown as area under the curve (AUC; arbitrary units). Data are shown as means \pm SE (n = 3–9). *Statistically significant difference between WKY and SHR.
tion of the signaling pathways involving the stimulation of adenylate cyclase. It is tempting to speculate that in SHR the increased endothelial production of prostacyclin (20–22) contributes to the preservation of blood fluidity, especially considering the elevated number of circulating platelets, but at the expense of being an EDCF in arteries containing smooth muscle cells lacking functional IP receptors. The molecular origin of this specific alteration in IP receptor-dependent relaxations remains to be identified. Nevertheless, mice knockout for the IP receptor (7, 60) and human patients with a dysfunctional prostacyclin IP receptor mutation (5) show accelerated atherothrombosis, indicating that an imbalance between vasconstrictor/relaxing and thrombogenic/antithrombogenic prostaglandins is of major importance in the generation of cardiovascular disease.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 10. Effects of prostacyclin (10⁻⁸ to 10⁻⁶ M) and beraprost (10⁻⁸ to 10⁻⁶ M) on ADP (10 or 30 μM)-induced platelet aggregation in 3-, 6-, and 15-mo-old WKY. Data are shown as means ± SE (n = 3–10).
Fig. 11. Effects of prostacyclin (10^{-8} to 10^{-6} M) and beraprost (10^{-9} to 10^{-6} M) on ADP (10 or 30 μM)-induced platelet aggregation in 3-, 6-, and 15-mo-old SHR. Data are shown as means ± SE (n = 3–10). *Statistically significant difference between SHR and WKY (shown in Fig. 10).
Fig. 12. Effects of CAY-10441 (CAY, 0.3 and 1 μM) plus prostacyclin (PGI2) and beraprost-induced inhibition of ADP-induced platelet aggregation in 3-, 6-, and 15-mo-old WKY. Data are shown as means ± SE (n = 3–10). *Group ADP plus prostacyclin (or beraprost) is significantly different from the group ADP alone and the group ADP plus CAY-10441 (1 μM) plus prostacyclin (or beraprost).


Fig. 13. Effects of CAY-10441 (CAY, 0.3 and 1 μM) on prostacyclin (PGI2)- and beraprost-induced inhibition of ADP-induced platelet aggregation in 3-, 6-, and 15-mo-old SHR. Data are shown as means ± SE (n = 3–10). *Group ADP plus prostacyclin (or beraprost) is significantly different from the group ADP alone and the group ADP plus CAY-10441 (1 μM) plus prostacyclin (or beraprost).

Fig. 14. Effect of forskolin (10^{-7} to 10^{-5} M) on ADP (10 or 30 μM)-induced platelet aggregation in 3-, 6-, and 15-mo (m)-old WKY (top) and SHR (bottom). Data are shown as means ± SE (n = 3–6). *Statistically significant difference in the effect of forskolin between WKY and SHR.


