PGH$_2$-TxA$_2$-receptor blockade restores vasoreactivity in a new rodent model of genetic hypertension

HIDEYUKI SUZUKI, HIROYUKI IKEZAKI, DENNIS HONG, AND ISRAEL RUBINSTEIN
Department of Medicine, University of Illinois at Chicago, and West Side Department of Veterans Affairs Medical Center, Chicago, Illinois 60612

Suzuki, Hideyuki, Hiroyuki Ikezaki, Dennis Hong, and Israel Rubinstein. PGH$_2$-TxA$_2$-receptor blockade restores vasoreactivity in a new rodent model of genetic hypertension. J Appl Physiol 88: 1983–1988, 2000.—The purpose of this study was to determine whether activation of prostaglandin H$_2$-thromboxane A$_2$ (PGH$_2$-TxA$_2$) receptors impedes vasodilation in the in situ peripheral microcirculation of spontaneously hypertensive hamsters, a new rodent model of high-renin genetic hypertension. Using intravital microscopy, we found that vasodilation elicited by suffusion of acetylcholine and vasoactive intestinal peptide (VIP), two neurotransmitters localized in perivascular nerves in the peripheral circulation, on the in situ cheek pouch was significantly attenuated in spontaneously hypertensive hamsters relative to age- and genetically matched normotensive hamsters (P < 0.05). However, nitroglycerin-induced vasodilation was similar in both groups. Pretreatment with SQ-29548, a selective and potent PGH$_2$-TxA$_2$-receptor antagonist, restored acetylcholine- and VIP-induced vasodilation in spontaneously hypertensive hamsters. SQ-29548 had no significant effects on resting arteriolar diameter and on nitroglycerin-induced vasodilation in both groups. SQ-29548 slightly but significantly potentiated VIP- but not acetylcholine-induced vasodilation in normotensive hamsters. Collectively, these data indicate that activation of PGH$_2$-TxA$_2$ receptors impedes agonist-induced vasodilation in the in situ cheek pouch of spontaneously hypertensive hamsters. We suggest that this model is suitable for studying the role of prostanoids in mediating vasomotor dysfunction observed in genetic hypertension.

essential hypertension; microcirculation; vasomotor tone; nitric oxide; acetylcholine; vasoactive intestinal peptide; nitroglycerin; prostaglandins; SQ-29548

DESPITE RECENT ADVANCES IN medical technology and therapeutics, essential hypertension remains a major public health problem in the US (41). Hence, there is an urgent need to develop new drugs to treat essential hypertension based on the proposed mechanisms underlying its pathophysiology (2, 3, 17, 25, 27, 43).

Essential hypertension is characterized by an increase in peripheral vascular resistance (3, 18, 25, 43). Present concepts suggest that this process is mediated, in part, by overproduction of contracting factors, such as prostaglandin H$_2$ (PGH$_2$) and thromboxane A$_2$ (TxA$_2$), and by microvascular endothelium in various microvascular beds that counteract vasorelaxation (1, 4, 5, 7, 13, 16, 19, 29). For instance, activation of PGH$_2$-TxA$_2$ receptors has been shown to attenuate endothelium-dependent vasodilation in the spontaneously hypertensive rat (SHR) (1, 5, 7, 13, 16, 19, 29). This model of normal renin genetic hypertension is used extensively to study hypertension pathogenesis (27, 43). However, the implications of microvascular dysfunction observed in SHR for other models of genetic hypertension are uncertain. This notion is important for proper interpretation of preclinical efficacy data reported on novel antihypertensive drugs being developed for clinical use (27, 41, 43).

To this end, we recently characterized and studied a new rodent model of high-renin genetic hypertension, the spontaneously hypertensive hamster, and its age- and genetically matched control (8, 30, 35, 39, 42, 45). We found that vasodilation elicited by acetylcholine and vasoactive intestinal peptide (VIP), two structurally distinct neurotransmitters localized in perivascular nerves of various species including hamster (9, 12, 14, 15, 26, 33, 34), but not by nitroglycerin, are blunted in the in situ cheek pouch of spontaneously hypertensive hamsters relative to normotensive controls (39). However, the mechanisms underlying this response were not elucidated.

Hence, the purpose of this study was to begin to address this issue by determining whether activation of PGH$_2$-TxA$_2$ receptors, which elicits contraction of cheek pouch arterioles in normotensive hamsters (6, 10, 11, 21, 28, 32, 36, 37, 44), impedes vasodilation elicited by acetylcholine and VIP in the in situ cheek pouch of spontaneously hypertensive hamsters.

METHODS
Preparation of Animals

Twenty-week-old male spontaneously hypertensive hamsters (n = 28; 142 ± 2 g body wt; mean arterial pressure, 164 ± 3 mmHg) and age- and genetically matched normotensive controls (n = 28; 152 ± 3 g body wt; mean arterial pressure, 97 ± 2 mmHg) were obtained from Canadian Hybrid Farms (Halls Harbour, Nova Scotia, Canada). These animals were previously used in our laboratory (8, 30, 35, 39, 42, 45). Hamsters were anesthetized with pentobarbital sodium (6 mg/100 g body wt, ip). A tracheostomy was performed to facilitate spontaneous breathing. A femoral vein was cannulated to inject supplemental anesthesia during the experiment (2–4 mg/100 g body wt$^{-1}$·h$^{-1}$). A femoral artery was cannulated to monitor systemic arterial pressure and heart rate, which did not change significantly during the
experiments. Body temperature was monitored and maintained constant (37–38°C) throughout the experiment via a feedback controller and a heating pad.

To visualize the microcirculation of the cheek pouch, we used a method previously described in our laboratory (20, 21, 24, 30–32, 35, 37–39). Briefly, the left cheek pouch was spread over a plastic base plate, and an incision was made in the overlying skin to expose the cheek pouch membrane. The avascular connective tissue layer of the membrane was carefully cut away, and an upper plastic chamber was positioned over the base plate. This arrangement forms a triple-layered complex: the base plate, the upper chamber, and the cheek pouch membrane exposed between the two plates. The chamber contains the suffusion fluid and is connected via a three-way valve to a reservoir that allows continuous suffusion of the cheek pouch with warm (37–38°C) bicarbonated buffer (pH 7.4) at a rate of 2 ml/min. The buffer is bubbled continuously with 95% N₂-5% CO₂. The chamber is also connected via a three-way valve to an infusion pump (Sage Instruments, Boston, MA) for controlled administration of drugs into the suffusate.

Determination of Arteriolar Diameter

The cheek pouch microcirculation was visualized with a microscope (long working distance objective ×4; eyepiece ×10; Nikon, Tokyo, Japan) coupled to a 100-W mercury light source. The microscope image was projected through a low-light television camera (Panasonic TR-124 MA, Matsushita Communication Industrial, Yokohama, Japan) onto a video screen (Panasonic). The inner diameter of second-order arterioles (48 ± 1 µm) was determined during the experiment from the video display of the microscopic image by using a videomicrometer with resolution of ±0.5 µm (VIA 100; Boeckler Instruments, Tucson, AZ). This system was calibrated routinely against a precise line-width standard, and the measurements were found to have <1% error. In each animal, the same arteriolar segment was used to measure changes in diameter during the experiment (20, 21, 24, 30–32, 35, 37–39).

Experimental Protocols

Effects of SQ-29548 on acetylcholine-induced vasodilation. Our laboratory has previously shown that vasodilation elicited by acetylcholine, an endothelium- and nitric oxide (NO)-dependent vasodilator in the cheek pouch (31, 32, 37, 39, 40), is attenuated in the cheek pouch of spontaneously hypertensive hamsters relative to age- and genetically matched normotensive controls (39). The purpose of these studies was to determine whether SQ-29548, a selective and potent PGH₂-TxA₂-receptor antagonist (22, 23, 37), restores acetylcholine-induced vasodilation in spontaneously hypertensive hamsters. The cheek pouch was suffused with bicarbonated buffer (pH 7.4) for 30 min (equilibration period). Then two concentrations of acetylcholine (0.1 and 1.0 µM) were suffused on the cheek pouch of hypertensive and normotensive hamsters for 7 min each in a random order. At least 30 min elapsed between subsequent suffusions of acetylcholine. When a drug was suffused on the cheek pouch, we determined whether SQ-29548, a selective and potent PGH₂-TxA₂-receptor antagonist, impedes vasodilation elicited by nitroglycerin, a NO donor that elicits endothelium-independent vasodilation in the cheek pouch (20, 31, 32, 37, 39), in spontaneously hypertensive hamsters. After the equilibration period, two concentrations of nitroglycerin (0.1 and 1.0 µM) were suffused on the cheek pouch of hypertensive and normotensive hamsters for 7 min each in a random order. At least 30 min elapsed between subsequent suffusions of nitroglycerin. Once suffusion of nitroglycerin was stopped and arteriolar diameter returned to baseline, SQ-29548 (1.0 µM) was suffused 30 min before and during repeated suffusions of nitroglycerin (0.1 and 1.0 µM). In preliminary studies, we determined that repeated suffusions of nitroglycerin (0.1 and 1.0 µM) for 7 min at 30-min intervals were associated with reproducible results. The concentrations of nitroglycerin used in these studies are based on previous studies in our laboratory (24, 38, 39).

Effects of SQ-29548 on nitroglycerin-induced vasodilation. The purpose of these studies was to determine whether SQ-29548 impedes vasodilation elicited by nitroglycerin, a NO donor that elicits endothelium-independent vasodilation in the cheek pouch (20, 31, 32, 37, 39), in spontaneously hypertensive hamsters. After the equilibration period, two concentrations of nitroglycerin (0.1 and 1.0 µM) were suffused on the cheek pouch of hypertensive and normotensive hamsters for 7 min each in a random order. At least 30 min elapsed between subsequent suffusions of nitroglycerin. Once suffusion of nitroglycerin was stopped and arteriolar diameter returned to baseline, SQ-29548 (1.0 µM) was suffused 30 min before and during repeated suffusions of nitroglycerin (0.1 and 1.0 µM). In preliminary studies, we determined that repeated suffusions of nitroglycerin (0.1 and 1.0 µM) for 7 min at 30-min intervals were associated with reproducible results. The concentrations of nitroglycerin used in these studies are based on previous studies in our laboratory (20, 31, 32, 37, 39).

Drugs

Acetylcholine was obtained from Sigma Chemical (St. Louis, MO). Human VIP was obtained from American Peptide (Sunnyvale, CA). Nitroglycerin was obtained from American Reagent Laboratories (Shirley, NY). SQ-29548 was obtained from Research Biochemicals International (Natick, MA). The drug was dissolved in ethanol as a 1 mM stock solution and diluted in saline to the desired concentration. All other drugs were dissolved and diluted in saline to the desired concentrations on the day of the experiment.

Data and Statistical Analyses

When a drug was suffused on the cheek pouch, we determined the maximal change in arteriolar diameter and used it as the response to that drug in each animal. Arteriolar diameter was expressed as the ratio of experimental to control diameter, with control diameter normalized to 100%, to account for intra- and interanimal variability. Data are expressed as the ratio of experimental to control diameter, with control diameter normalized to 100%, to account for intra- and interanimal variability. Data are...
expressed as means ± SE except for body weight data, which are expressed as means ± SD, because these data are not used for comparison between experimental groups. Statistical analysis was performed by using repeated-measures ANOVA with Newman-Keuls multiple-range post hoc test to detect values that were different from control values. A P value < 0.05 was considered statistically significant. The n value is given as the number of experiments, with each experiment representing a separate animal.

RESULTS

Effects of SQ-29548 on Acetylcholine-induced Vasodilation

Suffusion of acetylcholine (0.1 and 1.0 µM) on the cheek pouch of normotensive hamsters elicited significant concentration-dependent vasodilation (13 ± 1 and 27 ± 5% increase from baseline diameter, respectively; Fig. 1A; each group, n = 4 animals; P < 0.05). By contrast, acetylcholine (0.1 and 1.0 µM)-induced vasodilation was significantly attenuated in spontaneously hypertensive hamsters (6 ± 2 and 13 ± 4% increase from baseline diameter, respectively; Fig. 1B; each group, n = 4 animals; P < 0.05 compared with normotensive hamsters). Pretreatment with SQ-29548 (1.0 µM) had no significant effects on acetylcholine (0.1 and 1.0 µM)-induced vasodilation in the cheek pouch of normotensive hamsters (15 ± 1 and 28 ± 2% increase from baseline diameter, respectively; Fig. 1A; each group, n = 4 animals; P > 0.05 compared with acetylcholine in the absence of SQ-29548). However, SQ-29548 (1.0 µM) restored acetylcholine-induced vasodilation in spontaneously hypertensive hamsters (15 ± 2 and 21 ± 4% increase from baseline diameter, respectively; Fig. 1B; each group, n = 4 animals; P < 0.05 compared with acetylcholine in the absence of SQ-29548).

Effects of SQ-29548 on VIP-induced Vasodilation

Suffusion of VIP (0.05 and 0.1 nmol) on the cheek pouch of normotensive hamsters elicited significant concentration-dependent vasodilation (5 ± 1 and 10 ± 1% increase from baseline diameter, respectively; Fig. 2A; each group, n = 4 animals; P < 0.05). By contrast, VIP (0.05 and 0.1 nmol)-induced vasodilation was significantly attenuated in spontaneously hypertensive hamsters relative to normotensive controls (2 ± 1 and 5 ± 1% increase from baseline diameter, respectively; Fig. 2B; each group, n = 4 animals; P < 0.05 compared with normotensive hamsters). Pretreatment with SQ-29548 (1.0 µM) significantly potentiated VIP (0.05 and 0.1 nmol)-induced vasodilation in the cheek pouch of normotensive hamsters (9 ± 1 and 13 ± 1% increase from baseline diameter, respectively; Fig. 2A; each group, n = 4 animals; P < 0.05 compared with VIP in the absence of SQ-29548). In addition, SQ-29548 (1.0 µM) restored VIP-induced vasodilation in spontaneously hypertensive hamsters (11 ± 1 and 16 ± 1% increase from baseline diameter, respectively; Fig. 2B; each group, n = 4 animals; P < 0.05 compared with VIP in the absence of SQ-29548).

Effects of SQ-29548 on Nitroglycerin-induced Vasodilation

Suffusion of nitroglycerin (0.1 and 1.0 µM) elicited significant concentration-dependent vasodilation of similar magnitude in the cheek pouch of normotensive and spontaneously hypertensive hamsters (19 ± 3 and 31 ± 2, and 17 ± 1 and 31 ± 1% increase from baseline, respectively; Fig. 3; each group, n = 4 animals; P < 0.05 compared with baseline). Pretreatment with SQ-29548 (1.0 µM) had no significant effects on nitroglycerin (0.1 and 1.0 µM)-induced responses in normotensive and spontaneously hypertensive hamsters (17 ± 1 and 26 ± 2, and 20 ± 2 and 36 ± 8% increase from baseline, respectively; Fig. 3; each group, n = 4 animals; P > 0.05 compared with nitroglycerin in the absence of SQ-29548).

DISCUSSION

The new finding of this study is that activation of PGH₂-TxA₂ receptors impedes vasodilation elicited by
acetylcholine and VIP, but not by nitroglycerin, in the in situ cheek pouch of spontaneously hypertensive hamsters, a newly characterized rodent model of high-renin genetic hypertension (42). This conclusion is based on the observation that SQ-29548, a selective and potent PGH2-TxA2-receptor antagonist (21–23, 28), restored vasodilation in cheek pouch microvessels elicited by acetylcholine and VIP, two structurally and functionally distinct vasodilators in the cheek pouch microcirculation of spontaneously hypertensive hamsters (24, 39). The salutary effects of SQ-29548 were not related to a nonspecific response(s) because SQ-29548 had no significant effects on resting arteriolar diameter and nitroglycerin-induced vasodilation in normotensive and spontaneously hypertensive hamsters.

Taken together, these data suggest that suffusion of acetylcholine and VIP on cheek pouch microvessels of spontaneously hypertensive hamsters is associated with activation of PGH2-TxA2 receptors, which, in turn, impedes agonist-induced vasodilation. This notion is supported by the study of Suzuki et al. (37), who showed that suffusion of SQ-29548 on the cheek pouch of normotensive hamsters at a concentration similar to that used in this study abrogates the attenuating effects of smokeless tobacco extract on acetylcholine- and bradykinin-induced vasodilation. The results of this study also imply that production and/or responsiveness of resistance arterioles to endothelium-derived vasodilators, such as NO, is preserved in the cheek pouch of spontaneously hypertensive hamsters provided that PGH2-TxA2 receptors in microvessels are blocked (6, 10, 11, 21, 28, 37, 44). Clearly, additional studies using biochemical, molecular, and cell biology techniques are warranted to elucidate the mechanisms underlying the interactions between acetylcholine, VIP, and PGH2-TxA2 receptors in microvascular endothelium in spontaneously hypertensive hamsters.

Fig. 2. Effects of suffusion of vasoactive intestinal peptide (VIP) on arteriolar diameter in the in situ cheek pouch of normotensive (A) and hypertensive (B) hamsters in absence and presence of SQ-29548. Values are means ± SE; each group, n = 4. *P < 0.05 compared with baseline. †P < 0.05 compared with VIP alone.

Fig. 3. Effects of suffusion of nitroglycerin on arteriolar diameter in the in situ cheek pouch of normotensive (A) and hypertensive (B) hamsters in absence and presence of SQ-29548. Values are means ± SE; each group, n = 4. *P < 0.05 compared with baseline.
suffusion of PGH₂ elicits potent vasoconstriction in the cheek pouch of normotensive hamsters that exceeded that evoked by norepinephrine. In addition, Durán and Dillon (6) showed that platelet-activating-factor-induced vasoconstriction is mediated by TXA₂ in this organ. Similarly, Shepherd and Duling (36) showed that inosine evokes vasoconstriction in the cheek pouch of normotensive hamsters by releasing both TXA₂ and histamine from perivascular mast cells. Whether acetylcholine and VIP activate perivascular mast cells and/or other structural and migrant cells in the cheek pouch microcirculation of spontaneously hypertensive hamsters to elaborate PGH₂ and/or TXA₂ remains to be determined.

The hamster cheek pouch is an established model to investigate the mechanisms regulating vasomotor tone, including the role of NO, VIP, and products of arachidonic acid metabolism, in the in situ peripheral microcirculation (6, 10, 11, 20, 21, 24, 28, 31, 32, 35–40). Previous studies showed that acetylcholinesterase and VIP are localized in perivascular nerves in various vascular beds of the hamster, including the cheek pouch (Refs. 26, 34; S. Gulbenkian and I. Rubinstein, unpublished observations). We and other investigators have shown that successive suffusions of acetylcholine, VIP, and nitroglycerin on the cheek pouch at appropriate time intervals are associated with reproducible vasodilation in the absence of tachyphylaxis (20, 21, 24, 28, 31, 32, 35, 37–40, 44). Consequently, the effects of these compounds on vasomotor tone can be tested repeatedly in the same microvascular bed so that each animal serves as its own control. This, in turn, reduces the number of animals required per experiment and facilitates data analysis.

Previous studies showed that NO and/or a NO-containing compound(s) mediates, in part, the vasorelaxant effects of acetylcholine in the cheek pouch of normotensive hamsters (28, 37). By contrast, Onyüksel et al. (24) showed recently that the vasorelaxant effects of VIP in this organ are NO independent. Suzuki et al. (39) showed that vasodilation elicited by acetylcholine and VIP in the in situ cheek pouch of spontaneously hypertensive hamsters is blunted. These data suggest that the mechanism(s) underlying the impaired in vivo responses of resistance arterioles in this model is not related to inherent L-arginine/NO biosynthetic pathway dysfunction. Rather, activation of PGH₂–TXA₂-receptor-dependent biological cascade in the microcirculation may constitute an important pathway opposing acetylcholine- and VIP-induced vasodilation in this model of genetic hypertension. Similar observations have been reported in certain vascular beds of SHR's, a rodent model of normal-renin genetic hypertension (1, 5, 13, 16, 19, 29). We propose that therapeutic interventions which counteract activation of PGH₂–TXA₂ receptors in the peripheral microcirculation could be beneficial in the treatment of essential hypertension (2, 3, 17, 25, 43).

In summary, we found that activation of PGH₂–TXA₂ receptors impedes vasodilation elicited by acetylcholine and VIP, but not by nitroglycerin, in the in situ cheek pouch of spontaneously hypertensive hamsters, a new rodent model of high-renin genetic hypertension. We suggest that this model is suitable for studying the role of prostanoids in mediating vasomotor dysfunction observed in genetic hypertension.

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Address for reprint requests and other correspondence: I. Rubinstein, Dept. of Medicine (M/C 787), Univ. of Illinois at Chicago, 840 S. Wood St., Chicago, IL 60612–7323 (E-mail: IRubinst@uic.edu).

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