Low-amplitude pulses to the circulation through periodic acceleration induces endothelial-dependent vasodilatation

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Uryash A, Wu H, Bassuk J, Kurlansky P, Sackner MA, Adams JA. Low-amplitude pulses to the circulation through periodic acceleration induces endothelial-dependent vasodilatation. J Appl Physiol 106: 1840–1847, 2009. First published March 26, 2009; doi:10.1152/japplphysiol.91612.2008.—Low-amplitude pulses to the vasculature increase pulsatile shear stress to the endothelium. This activates endothelial nitric oxide (NO) synthase (eNOS) to promote NO release and endothelial-dependent vasodilatation. Descent of the dicrotic notch on the arterial pulse waveform and a-to-b ratio (a/b; where a is the height of the pulse amplitude and b is the height of the dicrotic notch above the end-diastolic level) reflects vasodilator (increased a/b) and vasoconstrictor effects (decreased a/b) due to NO level change. Periodic acceleration (pGz) (motion of the supine body head to foot on a platform) produces systemic additional pulsatile shear stress. The purpose of this study was to determine whether or not pGz applied to rats produced endothelial-dependent vasodilatation and increased NO production, and whether the latter was regulated by the Akt/phosphatidylinositol 3-kinase (PI3K) pathway. Male rats were anesthetized and instrumented, and pGz was applied. Sodium nitroprusside, N⁶-nitro-l-arginine methyl ester (l-NNAME), and wortmannin (WM; to block Akt/PI3K pathway) were administered to compare changes in a/b and mean aortic pressure. Descent of the dicrotic notch occurred within 2 s of initiating pGz. Dose-dependent increase of a/b and decrease of mean aortic pressure took place with SNP. l-NNAME produced a dose-dependent rise in mean aortic pressure and decrease of a/b, which was blunted with pGz. In the presence of WM, pGz did not decrease aortic pressure or increase a/b. WM also abolished the pGz blunting effect on blood pressure and a/b of l-NNAME-treated animals. eNOS expression was increased in aortic tissue after pGz. This study indicates that addition of low-amplitude pulses to circulation through pGz produces endothelial-dependent vasodilatation due to increased NO in rats, which is mediated via activation of eNOS, in part, by the Akt/PI3K pathway.

ADDED LOW-AMPLITUDE PULSES TO THE VASCULATURE were generated with periodic acceleration (pGz). pGz is produced by a motorized platform that repetitively moves the horizontally oriented body sinusoidally in a head to foot direction. Inertia of fluid as the body accelerates and decelerates adds a small-amplitude pulse to the circulation that is superimposed on the natural pulse, increasing pulsatile shear stress to the endothelium (3, 6, 7). In large-animal models, increased pulsatile shear stress activates endothelial nitric oxide (NO) synthase (eNOS) to release NO into the circulation, which, in turn, induces endothelial-dependent pulmonary and systemic vasodilatation, as well as increasing organ blood flows (3, 5, 6). pGz applied to anesthetized swine increases serum nitrite, which is a qualitative marker for the release of NO (4, 44). pGz leads to a phosphorylation of eNOS that is correlated with a phosphorylation of Akt in endothelial cells (48). The release of NO into the circulation with pGz has been shown to be physiologically meaningful and long lasting in a sheep model of asthma (1). Additionally, pGz applied to human subjects increases brachial flow-mediated vasodilation and induces release of NO, which is comparable to light to moderate exercise (36, 47).

Because NO synthesized by eNOS is metabolized within 4 s, NO cannot be measured directly in the bloodstream. Instead, serum nitrite, a metabolite, is measured as a qualitative marker of NO release. The same increased values of serum nitrite in the nanomole range that occur with increased shear stress during flow-mediated dilation or aerobic exercise do not produce immediate vasodilatation when infused into the circulation at such concentrations in humans (30, 44, 47). Therefore, in this investigation, we relied on the vasodilator properties of NO to assess effectiveness of pGz. It has long been recognized that nitrates produce descent of the dicrotic notch on the descending limb of the digital pulse waveform of humans (13). This phenomenon is attributed to vasodilatation of the resistance vessels, thereby lengthening the arterial pulse wave reflection time (26). In humans, descent of the dicrotic notch of digital and aortic pulse waves has been observed with nitrate and albuterol administration, as well as with exercise (10, 26, 29, 39, 42, 46). This finding is mediated through the action of NO, since inhibition of NO synthesis with N⁶-nitro-l-arginine methyl ester (l-NNAME) or N⁶-monomethyl-l-arginine prevents descent of the dicrotic notch after acetylcholine or albuterol administration in rabbits and humans, respectively (26, 52).

Our purpose was to assess whether pGz applied to rats serves as a means of producing endothelial-dependent vasodilatation and to test whether such an effect is mediated, in part, via the well-known shear stress induced Akt/phosphatidylinositol 3-kinase (PI3K) pathway for NO production (13, 55, 57).

MATERIALS AND METHODS

Animal Preparation

These studies were approved by the Mount Sinai Medical Center Institutional Animal Care and Use Committee and comply with the Animal Welfare Act.

Albino rats (n = 60), weighing between 300 and 320 g (Charles River Laboratories, Wilmington, MA), were anesthetized with intramuscular ketamine (60 mg/kg)/xylazine (10 mg/kg) until a surgical plane of anesthesia was obtained and supplemented throughout the experiment, along with support doses as necessary. A tracheotomy was performed, and a 14G endotracheal tube inserted. A size 2F Millar Mikro-Tip pressure catheter was inserted into the right carotid
artery to measure ascending aortic pressure and dicrotic notch position (Millar Instruments, Houston, TX). The a-to-b ratio (a/b) was computed from the aortic pressure pulse, where a is the height of the pulse amplitude divided by b, the height of the dicrotic notch above the end-diastolic level. An increase of the a/b signifies vasodilatation, and a decrease vasoconstriction. A 24-gauge catheter was placed into the tail vein for administration of fluids and drugs.

The animals were maintained at 38°C with a thermostatically controlled warming pad. The animals were placed on the motion platform in the supine position with the front and hind legs taped securely to the table and the rat’s body closely coupled to the platform. The tracheotomy tube was connected to a small-animal mechanical ventilator delivering inspired O₂ fraction of 0.21 (model CIV-101, Columbus Instruments, Columbus, OH). This was set to 70–75 breaths/min with tidal volumes of 4.0–4.5 ml, such that arterial PCO₂ was maintained at 38 Torr.

To assess the contribution of the shear stress-induced pathway Akt/PI3K on the pGz-induced, endothelial vasodilatation, wortmannin (WM), a specific inhibitor of PI3K, was used. Cumulative dose-response curves in terms of the a/b and mean aortic pressure were obtained from intravenous administration of WM, and without pGz. Animals were randomized to WM (15 μg/kg) and pGz, of 360 cpm (experimental groups, n = 6) with a Gz ± 2.9 m/s² held constant and the same dose of WM without pGz (control group, n = 6). Four rats were also injected with l-NAME (4 mg/kg) after WM and pGz (WM/pGz/l-NAME group).

Experimental Design

Aortic pressure and a/b changes. After surgical preparation, the anesthetized rats were connected to a mechanical ventilator. Baseline (BL) measurements of hemodynamic parameters were obtained on all animals. In the first group of animals (n = 6), 12.5 μg/kg of sodium nitroprusside (SNP) was administered through the tail vein. A second group of animals (n = 24) received l-NAME (Sigma Chemicals, St. Louis, MO) infused into the tail vein over a 10-min period, for a total cumulative dose of 6 mg/kg. A third group of rats (n = 6), 12.5 to 71 mmHg (SD 11) (360-cpm pGz) and 112 (SD 3) to 85 mmHg (SD 5) at BL and 30 min, respectively. P < 0.05). Similarly, 360- and 600-cpm pGz produced a decrease in MAP from 112 (SD 6) to 71 mmHg (SD 11) (360-cpm pGz) and 112 (SD 3) to 85 mmHg (SD 5) (600-cpm pGz) at BL and 30 min, respectively (P < 0.05, BL vs. pGz). SNMP produced a dose-dependent decrease in MAP, and l-NAME a dose-dependent increase, as expected (data not shown).

Effects of pGz, l-NAME, and SNP on Mean Aortic Pressure, HR, and Aortic Pressure Wave

Mean HR and mean arterial pressure (MAP) did not significantly differ among groups at BL. pGz at all frequencies tested did not produce a significant change in HR compared with BL values. Mean HR at BL in controls and pGz, of 180, 360, and 600 cpm were 315 (SD 8), 314 (SD 11), 315 (SD 17), and 314 beats/min (SD 20), respectively (P = nonsignificant). The application of pGz at all frequencies decreased MAP within 5 min of its administration, but the a/b increased by 25–50% from BL, within 2 s. MAP progressively decreased during 30 min after pGz, pGz of 180 cpm administration decreased MAP from BL of 111 (SD 7) to 98 mmHg (SD 6) (P < 0.05). Similarly, 360- and 600-cpm pGz produced a decrease in MAP from 112 (SD 6) to 71 mmHg (SD 11) (360-cpm pGz) and 112 (SD 3) to 85 mmHg (SD 5) (600-cpm pGz) at BL and 30 min, respectively (P < 0.05, BL vs. pGz). SNMP produced a dose-dependent decrease in MAP, and l-NAME a dose-dependent increase, as expected (data not shown).

pGz at all frequencies induced added pulses visible on the aortic pulse waveform of 1–3 mmHg. These added pulses occurred at peaks and troughs of acceleration of the motion platform (Fig. 1). The descent of the dicrotic notch pressure wave was made to be 2 s. The level of actin was used as loading control. The image was analyzed using Image J 1.36b (National Institutes of Health).

Effects of pGz, on l-NAME and comparison to SNP. Cumulative dose-response curves in terms of the a/b and mean aortic pressure were obtained from intravenous administration of l-NAME (Sigma Chemical) with different frequencies of pGz. Animals were randomized to pGz, of 180, 360, and 600 cpm (experimental groups) with Gz ± 2.9 m/s² held constant. Rats either were subjected to one frequency for 30 min (pGz–30 min) or were placed on a platform for the same time period without applying pGz (control). l-NAME (n = 24) was infused into the tail vein over a 10-min period to produce cumulative doses of 0.5, 1, 2.5, 4, 5, and 6 mg/kg. In another group of animals, SNP (n = 6) was continuously infused through a light protected tail vein with cumulative doses of 0.5, 2.5, and 12.5 μg/kg.

The following were calculated for 30 s at the end of each period of BL, pGz, l-NAME, or SNP infusion: 1) mean HR, 2) mean aortic pressure, and 3) mean a/b.
but the overall downward descent was maintained throughout the pGz period.

\( a/b \)

SNP and pGz caused a dose-dependent increase in \( a/b \). At equivalent mean decrease of aortic pressure of 32% for SNP (3 \( \mu \)g/kg dose) and pGz (360 cpm), SNP increased \( a/b \) from 1.8 (SD 0.1) BL to 2.2 (SD 0.2) (25% change) \((P < 0.05, BL vs. SNP)\), whereas pGz of 360 cpm increased from 1.6 (SD 0.2) BL to 3.6 (SD 0.3) (129% change) \((P < 0.05, BL vs. pGz)\). The highest SNP (12.5 \( \mu \)g/kg) dose studied produced a 49% decrease in MAP, with a corresponding increase of \( a/b \) of 58% of BL \((P < 0.05, BL vs. SNP)\) (Fig. 2). Compared with pGz of 180 and 600 cpm, pGz of 360 cpm produced the largest increase in \( a/b \) from 1.6 (SD 0.2) BL to 3.6 (SD 0.3) \((P < 0.05, 360-cpm pGz vs. 180- and 600-cpm pGz)\). pGz of 360 cpm produced an increase in \( a/b \) of 129% after 30 min from BL, with a corresponding mean aortic pressure decrease of 37% \((P < 0.05, BL vs. pGz)\) (Fig. 2).

L-NAME infusion produced a dose-dependent and significant decrease in \( a/b \) (Fig. 2). The dose of L-NAME (2.5 mg/kg) studied increased MAP to 52% of BL, and correspondingly decreased \( a/b \) from BL 1.8 (SD 0.2) to 1.0 (SD 0.1) after L-NAME \((P < 0.05, BL vs. L-NAME)\).

Effects of pGz on a Dose-Response Curve of L-NAME

L-NAME produced a dose-dependent increase of mean aortic pressure and decrease in \( a/b \) in both control and pGz groups. pGz of 180, 360, and 600 cpm all blunted the L-NAME-induced rise of mean aortic pressure. Additionally, all pGz frequencies tested shifted the L-NAME dose response to the right, with a delayed dose-dependent increase of MAP by 2 min compared with control. The rate of MAP increase for control was 9.8 (SD 0.3), for 180-cpm pGz was 7.2 (SD 0.6), for 600-cpm pGz was 6.7 (SD 0.9), and for 360-cpm pGz was 5.6 mmHg/min (SD 1.2) \((P < 0.05, 360- and 600-cpm pGz vs. control)\). This blunting effect was most pronounced at 360-cpm pGz. Mean aortic pressure rise in control and 180-cpm pGz plateaued at a
dose of 2.5 mg/kg of L-NAME. In contrast, this plateau in MAP was reached at higher doses of L-NAME for pGz of 360 and 600 cpm. MAP plateau in pGz of 360 cpm was reached at 4 mg/kg of L-NAME and for pGz of 600 cpm at 3.0 mg/kg (P < 0.05, 360- and 600-cpm pGz vs. control). Thus the maximum attenuation of MAP increase at each dose of L-NAME was observed with pGz applied at 360 cpm (Fig. 3).

During the dose response to L-NAME, a/b significantly decreased in control in a dose-dependent manner from 2.1 (SD 0.2) to 1.0 (SD 0.1). In contrast, a/b, in animals receiving pGz of 360 cpm during L-NAME infusion, showed decrease from 3.6 (SD 0.3) and plateaued at 2.0 (SD 0.2) (P < 0.05, 360-cpm pGz vs. control) (Fig. 4).

Effects of WM on pGz and of pGz on L-NAME in Presence of WM

WM caused a dose-dependent increase in MAP from 104 (SD 4) to 139 mmHg (SD 5) and drop in a/b of 35% from BL (P < 0.05, WM vs. BL) in the control group. pGz did not decrease mean aortic pressure or increase a/b, when pGz of 360 cpm was administered together with WM (Fig. 5). Mean aortic pressure continued to rise in the presence of WM after the start of pGz from 139 mmHg (SD 5) to 151 mmHg (SD 11) (P < 0.05, pGz vs. BL). pGz did not modify a/b in WM-pretreated animals. The blood pressure blunting effects of pGz on l-NAME were diminished by pretreatment with WM. The WM/ pGz/l-NAME group showed an 84% increase from BL in mean aortic pressure compared with 54% in a group of pGz of 360 cpm and l-NAME alone (P < 0.05, WM/pGz/l-NAME vs. pGz/l-NAME) (Fig. 6).

eNOS

A single 1-h exposure to 360-cpm pGz increased protein level of eNOS 70% over control (n = 3 each group) (P < 0.05, 360-cpm pGz vs. control) (Fig. 7).

DISCUSSION

The present study demonstrates that, similarly to humans, swine, and sheep, application of pGz to rats produces added low-amplitude pulses to the vasculature and delivers additional pulsatility to the endothelium. This, consequently, induces endothelial-dependent vasodilatation with release of NO into the circulation. The pulses added with pGz generate pulse pressures of 1–3 mmHg, which are identical in magnitude to
were diminished by pretreatment with WM. The WM/pGz/L-NAME group showed a larger increase in arterial pressure and decrease of \( \text{alb} \) compared with the pGz/L-NAME group. \( P < 0.05 \) vs. pGz/L-NAME, 15 \( \mu \text{g/kg} \) WM, 360-cpm pGz, and 4 mg/kg L-NAME. Values are expressed as percent change from baseline with SD (n = 4).

Several investigators have used the relative height of the dicrotic notch as a simple, noninvasive, and sensitive method for NO bioactivity in rabbits using a photoplethysmographic ear pulse, or in humans using either arterial pressures or digital volume pulse (20, 22, 23, 25, 34, 38, 40, 49, 52). Weinberg et al. (52) have also found a dose-dependent decrease in the relative height of the dicrotic notch or \( \text{alb} \) after acetylcholine and SNP, both of which were abolished by L-NAME, and additionally confirmed an NO pathway-mediated effect. Others have found the rise of the \( \text{alb} \) in humans after nitroglycerin administration (11, 20, 22, 23, 25, 38, 40, 43). Our results are consistent and in close agreement with the aforementioned investigators.

The magnitude of descent of the dicrotic notch in our study depended on the frequency of pGz, and the duration and dose of the NO donor infused. Application of pGz in normal rats caused a 15- to 20-mmHg decrease in mean aortic pressure after 30 min. These data are similar to those previously reported for anesthetized pigs, where mean arterial systemic blood pressure decreased from 107 mmHg at BL to 88 mmHg during pGz, and returned to BL values after pGz was discontinued (4). SNP infusion produced similar changes in mean aortic pressure at doses between 1 and 2.5 \( \mu \text{g/kg} \). Despite similar changes in arterial pressure, increases of the \( \text{alb} \) differed. pGz caused an 80–110% increase in \( \text{alb} \), whereas SNP infusion produced only a 20% increase for the same decline in arterial pressure. Since the \( \text{alb} \) is a function of arterial wave reflection, the lesser increase of the \( \text{alb} \) with SNP compared with pGz can be interpreted as follows: 1) the major site of action of SNP was on large-conduit vessels and pGz on smaller resistance vessels; or 2) pGz also stimulated release of prostacyclin, which provided significant additional vasodilatation to cause the lengthening of the time of wave reflection (4). Nier et al. (42) showed, in rabbits, that the relative height of the dicrotic notch was dose dependently decreased by the cyclooxygenase

Detection of increased NO in the present study was based on a significant rise of the \( \text{alb} \) of the aortic pulse, decreased mean aortic pressure, attenuation of L-NAME-induced hypertension along with decreased \( \text{alb} \), and activation of eNOS in the aortic wall. The release of NO from eNOS in rats produced by pGz is consistent with prior observations from our laboratory (1, 4, 6, 39, 42). SNP, an endothelial-independent NO donor drug, also increased \( \text{alb} \) and decreased mean aortic pressure in the rats of the present study, but not to the same extent as pGz when adjustments were made for comparable declines in mean aortic pressure. As expected, inhibition of NO synthesis with L-NAME and WM increased mean aortic pressure and decreased the \( \text{alb} \).

Fig. 6. The effects of pretreatment with WM on pGz ability to blunt L-NAME-induced changes of MAP (A) and \( \text{alb} \) (B). Compared with control (*P < 0.05 vs. control/L-NAME), the blood pressure blunting effects of pGz on L-NAME were diminished by pretreatment with WM. The WM/pGz/L-NAME group showed a larger increase of arterial pressure and decrease of \( \text{alb} \) compared with the pGz/L-NAME group. *P < 0.05 vs. pGz/L-NAME, 15 \( \mu \text{g/kg} \) WM, 360-cpm pGz, and 4 mg/kg L-NAME. Values are expressed as percent change from baseline with SD (n = 4).

Fig. 7. Effect of 360-cpm pGz on aortic protein levels of endothelial NO synthase (eNOS). pGz treatment significantly increased level of eNOS in rat aorta, as indicated by relative positive value of +70% compared with control. Values are expressed as percent change from control with SD (n = 6). *P < 0.05.
inhibitor indomethacin. This effect was blocked by L-NAME, suggesting prostaglandin action to be mediated by cross talk with the NO pathway (49). It is not likely that the prostacyclin released with pGz accounts for the difference between pGz and SNP effects on the alb., since prostacyclin is a much weaker vasodilator. For example, in patients undergoing coronary artery bypass surgery, administration of SNP to lower mean aortic pressure to 10 mm Hg increased the alb only 18% (22). Thus our results suggest that SNP mainly dilates large conduit vessels, whereas pGz mainly dilates smaller resistance vessels. Additionally, since normal rats were used in these experiments, structural changes in conduit or resistance vessels cannot account for the observed findings.

pGz significantly attenuated the L-NAME-induced increased mean aortic pressure and the concomitant decrease of the alb. To obtain a similar increase in mean aortic pressure during L-NAME infusion, 360-cpm pGz treated animals required twice the L-NAME dose, presumably due to pGz increased NO production. pGz blunted the dose-dependent vasconstrictor effect of L-NAME, which was expressed in a slower increase in aortic mean pressure for the same relative dose received by the animal. Decrease in alb paralleled mean aortic pressure increases during L-NAME infusion (Fig. 6).

The mechanisms whereby either laminar or pulsatile shear stress increases release of NO into the circulation are complex and multifactorial (12, 53). In intact animals, laminar shear stress has been thoroughly investigated (19, 33, 51). However, there has been much less study of pulsatile shear stress alone in intact animals, with the exception of extracorporeal circulation, owing to the paucity of modalities to induce pulsatile shear stress in vivo (31, 54). Nakano et al. (41), in an extracorporeal model of pulsatile shear stress, found that eNOS activation is largely caused by tyrosine kinase-sensitive activation. In the present study, one such tyrosine kinase pathway, Akt/PI3K, was investigated by administration of WM that blocks this pathway. This agent blocked the effects of pGz on aortic pressure, abolished the increase of the alb, and diminished the blunting effect of pGz on action of L-NAME. These findings are consistent with pGz activation of the Akt/PI3K pathway as a stimulus to eNOS activity, the latter via induced pulsations with circumferential stretch on the vascular endothelium. Our results also agree with in vitro endothelial cell culture findings of other investigators, who showed that pulsatile shear stress upregulates eNOS activity via the Akt/PI3K pathway (32, 41, 45, 57).

From a clinical perspective, pGz has been used in adult human subjects, without side effects. In healthy adults, pGz acutely produced increased NO into the circulation, measured using the alb and plasma levels of nitrates and nitrites, after 20 pGz sessions over 31 days (21, 46). pGz chronically applied (20 sessions over 4 wk) to sedentary adults improved vascular endothelial function (as measured by brachial artery flow-mediated vasodilatation). Furthermore, in heart failure patients, pGz chronically applied (5 days/wk over 5 wk) increased 6-min walk distance and postischemic flow (a marker of endothelial function) (27). Our findings of increased eNOS protein expression after 1 h of pGz suggest that pGz upregulates eNOS, which, via NO production, can explain the observed improvement in endothelial function. The optimal frequency observed in our experiments with rats (360 cpm) differs from those used in human subjects (140 cpm). This difference may be related to the differences in intrinsic HR of the two species, but remains to be elucidated.

Optimal release of NO occurred at the 360-cpm setting for pGz, which is a frequency close to the intrinsic rat HR; thus the possibility that resonance might be contributing to our results was considered. There is no information about the rat aorta for added pulses, as occurs with pGz, but a study has been reported in sheep with added pulses produced by intra-aortic balloon counterpulsation on aortic wall energetics and damping. During counterpulsation, diastolic pulses at twice the harmonic of the fundamental with amplitude of ~40 mmHg are produced compared with the ~3 mmHg of pGz added pulses sweeping through the systolic and diastolic periods. Counterpulsation augmented pulses increase pulse wave velocity, decrease aortic compliance, but did not alter aortic damping. Resonance for the counterpulsation pulses was not measured (18). In the rat abdominal aorta, weak resonant frequencies were recorded at the second and third harmonic, with the third harmonic significantly greater than the second harmonic of the fundamental (56). In another study using the rat thoracic aorta, a well-defined resonant peak measured by forced oscillation was recorded at 20 Hz, which is approximately the third harmonic of the rat HR in the present study (9). The observations that perhaps resonance may play a role during pGz is of interest and perhaps would be better studied under in vivo conditions.

Increase in cardiac output could alter mean fluid shear and affect NO production, as occurs with exercise and as others have reported (15, 16, 28, 31, 37). We have not directly measured cardiac output in rats in response to pGz. In swine, we have shown a small 11% increase in cardiac output from BL values at a constant pGz frequency of 240 cpm and acceleration of ±3 m/s² (5). This small change in cardiac output may be an additional contributor to NO production, but also requires further investigation.

Improving endothelial output via a noninvasive methodology could have significant impact for cardiovascular preconditioning, postconditioning, and planned ischemia-reperfusion events, such as surgery. Future in vitro and in vivo experiments using this model and molecular biology techniques will be useful to further understand the mechanisms whereby increased low-level pulsatility, such as pGz, may confer long-term benefits for endothelial function and tissue repair during and after pathological ischemia-reperfusion injury and cardiovascular events.

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