Continuous release of vasodilator prostanoids contributes to regulation of resting forearm blood flow in humans

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Duffy, Stephen J., Binh T. Tran, Gishel New, Ronald N. Tudball, Murray D. Esler, Richard W. Harper, and Ian T. Meredith. Continuous release of vasodilator prostanoids contributes to regulation of resting forearm blood flow in humans. *Am. J. Physiol.* 274 (*Heart Circ. Physiol.* 43): H1174–H1183, 1998.—Continuous release of nitric oxide contributes to the maintenance of resting tone in the human forearm and coronary circulations; however, evidence for a similar role of vasodilator prostanoids such as prostacyclin is lacking. We examined whether continuous release of prostacyclin contributes to basal forearm blood flow. Flow was measured using venous occlusion plethysmography in 38 healthy volunteers [mean age 21.3 ± 2.5 yr (±SD); 13 female, 25 male] at rest, after administration of three incremental intra-arterial infusions of either the cyclooxygenase inhibitor aspirin or placebo, and before and after administration of the endothelium-dependent and -independent dilators acetylcholine (30 μg/min) and nitroprusside (1 μg/min). To assess the effect of aspirin on the production of prostacyclin, plasma 6-keto prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$; the stable metabolite of prostacyclin) was measured by simultaneous arterial and venous sampling. Aspirin produced a time- and dose-dependent reduction in forearm blood flow, resulting in a 32% decrease at the highest dose. The effect was maximal after 10 min. Flow at rest and after aspirin doses of 1, 3, and 10 mg/min was 2.6 ± 0.2, 2.3 ± 0.2, 2.1 ± 0.2, and 1.8 ± 0.2 ml·100 ml forearm tissue^{-1}·min^{-1}, respectively (means ± SE, $P < 0.001$). Commensurate with these data, the net forearm production of 6-keto-$PGF_{1\alpha}$ was 52.9 ± 16.4, 11.7 ± 8.6, 18.7 ± 8.5, and 12.0 ± 12.5 pg·100 ml forearm tissue^{-1}·min^{-1} for the respective doses ($P = 0.04$). No time-dependent reduction in flow was seen in subjects with vehicle infusion. Aspirin did not affect the responses to acetylcholine or nitroprusside. These data suggest that continuous release of prostacyclin plays a role in the maintenance of resting forearm blood flow. There appears to be a direct link between the reduction in flow with aspirin and inhibition of prostacyclin production.

aspirin; eicosanoids; vasodilation; vasoconstriction; regional blood flow

MANY SYSTEMIC AND LOCAL factors have been postulated to be important in the control of resting skeletal muscle blood flow. The sympathetic nervous system, local vasodilator metabolites, and myogenic factors are thought to be the main contributors to this regulation (40). O_2 tension and pH may play a role, and locally released ions and metabolites thought to be important include potassium, inorganic phosphate, lactate, and, in particular, adenosine (40).

Recently, considerable interest has been focused on the role of endothelium-derived factors in regulation of vascular tone both at rest and during changes in metabolic demand (46). Evidence suggests that continuous release of endothelium-derived nitric oxide contributes to the maintenance of resting blood flow in both skeletal muscle (45) and coronary vascular beds (37). Vallance et al. (45) demonstrated a 50% reduction in resting forearm blood flow with intra-arterial infusion of the nitric oxide inhibitor N^G-monomethyl-L-arginine. In the coronary circulation Quyyumi et al. (37) have shown that the same inhibitor reduced resting coronary artery caliber and blood flow while increasing coronary vascular resistance.

Endothelial cells also produce a number of other vasoactive factors including prostaglandins, the as-yet unidentified endothelium-derived hyperpolarizing factor, endothelin, and angiotensin II (27). The principal vascular prostanoid in humans is the evanescent vasodilator prostacyclin (PGI_2) (18), and the endothelium is its main source (32). Besides its vasodilator function, PGI_2 is also the most potent known endogenous inhibitor of platelet aggregation (18). PGI_2 is produced from arachidonic acid by a series of enzymes including cyclooxygenase (42). In experimental studies on the skeletal muscle circulation (hindlimb preparation), intra-arterial cyclooxygenase inhibitors, including indomethacin, reduce resting blood flow and increase vascular resistance by ~40% (3, 50). In humans, Kilbom and Wennmalm (25) demonstrated that although vasodilator prostanoids did not appear to be involved in the maintenance of basal blood flow, they did contribute to postischemic and metabolic vasodilation in the forearm. Recently, Wilson and Kapoor (49) confirmed the role of prostaglandins in exercise-induced vasodilation in human skeletal muscle vasculature using an intra-arterial infusion of indomethacin, and they also detected a contribution of prostaglandin release in maintaining basal blood flow. Earlier human investigations have utilized cyclooxygenase inhibitors such as indomethacin or acetylsalicylic acid (aspirin) in either enteral or intravenous preparations (4, 25, 26), which may be limited by large volumes of distribution, rapid metabolism, and systemic effects that may evoke neural reflex compensation (22). Thus, despite biological functions similar to nitric oxide, most previous investigations in human forearm and coronary circulations suggest that PGI_2 primarily contributes to the blood flow response associated with increased metabolic demand or ischemia.

The primary objective of this investigation was to determine whether continuous release of PGI_2 contributes to the maintenance of resting vascular tone, and

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thus tissue perfusion, in human skeletal muscle. We hypothesized that infusion of aspirin directly into the brachial artery of healthy humans would result in a dose-dependent reduction in forearm blood flow commensurate with a reduction in the forearm production of prostacyclin. If it could be demonstrated that endothelium-derived PGI_2 contributes to resting and stimulated skeletal muscle blood flow, this may have important implications for the use of cyclooxygenase inhibitors (such as nonsteroidal anti-inflammatory drugs) in diseases that impair the release or synthesis of endothelium-derived substances.

METHODS

Subjects. We studied 38 healthy volunteers with a mean age of 21.3 ± 2.5 yr (±SD; 13 female, 25 male) recruited by advertisement at the Monash University campus. All subjects were screened for cardiovascular risk factors, cardiovascular disease, or other major illness by medical history, physical examination, and fasting lipid profile. Subjects were excluded if they had any of the following: cardiovascular risk factors (including a past or present history of smoking and family history of ischemic heart disease), cardiovascular disease, major noncardiac disease, or any abnormality on physical examination (including a discrepancy of ≥10 mmHg of blood pressure between the upper limbs). Subjects taking vasoactive medications were also excluded. The study was approved by the Human Research Ethics Committee of Monash Medical Centre and the National Health and Medical Research Council of Australia, and all subjects gave their written, informed consent.

General methods. Subjects were asked to refrain from caffeine-containing food and drinks and from alcohol for 12 h before the study. Aspirin and other nonsteroidal anti-inflammatory drugs were forbidden for a week before the study.

A 20-gauge, 5-cm polyethylene catheter (Cook, Brisbane, Australia) was introduced under local anesthesia into the brachial artery of the nondominant upper limb. The arterial cannula served as an infusion port for vasoactive agents and enabled blood pressure to be monitored directly and continuously. In one protocol involving nine subjects, the catheter was also used for arterial blood sampling. In these subjects an 18-gauge, 12-cm polyethylene venous catheter (Cook) was also inserted under local anesthesia into the medial cubital vein of the same limb to allow blood sampling from the deep venous drainage of the forearm (8).

So that a stable baseline could be established, all subjects rested for at least 30 min after arterial line insertion before the first measurement was made. During this time isotonic glucose (5% dextrose) was infused at a rate of 0.4 ml/min intra-arterially (the same rate at which all drugs were subsequently infused).

Drug infusion protocol. Aspirin (Aspisol, graciously supplied by Bayer, Leverkusen, Germany), a well-known inhibitor of cyclooxygenase that irreversibly acetylates this enzyme (22), was infused via the brachial artery in three incremental doses of 1, 3, and 10 mg/min. These doses were calculated to achieve local plasma concentrations (assuming a forearm blood flow of 2.5 ml·100 ml forearm tissue^{-1}·min^{-1}) of 50, 150, and 500 μg/ml, respectively (39, 48), and were estimated to inhibit endothelial PGI_2 production by ~80, 95, and 100%, respectively (29). These estimates were based on bioassay PGI_2 inhibition data derived from human studies by Masotti et al. (29).

Acetylcholine chloride (Miochol, Iolab Pharmaceuticals, Sydney, Australia), an agent that causes vasodilation principally by release of endothelium-derived nitric oxide (45), was infused via the brachial artery for 5 min in a dose of 30 μg/min as previously described (30). Sodium nitroprusside (Faulding, Melbourne, Australia), a nitric oxide donor that results in direct vascular smooth muscle relaxation, was administered via the brachial artery for 5 min at a rate of 1 μg/min, as described in previous studies (30), to assess the response to an endothelium-independent vasodilator.

All drugs were diluted in an isotonic glucose solution (5% dextrose) and were infused at a rate of 0.4 ml/min using a syringe pump (Terumo, Tokyo, Japan), the same rate as the vehicle infusion at baseline. Subjects who received vehicle infusion instead of aspirin were told that they were receiving aspirin.

Experimental protocols. Four experimental protocols were used in this study. Initially, we sought to determine whether there was an effect of aspirin on resting forearm blood flow and to assess the time course of any such effect. Second, we sought to establish a dose-response curve. We then examined the mechanism of action of aspirin by timed arteriovenous sampling for the stable PGI_2 metabolite 6-keto prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) (15, 38), norepinephrine, and blood gases. Finally, we compared the effect of aspirin infusion against placebo (vehicle infusion) and determined whether aspirin affected the responses to endothelium-dependent and -independent vasodilators.

Time course. This protocol was designed to assess the time course of the effect of a single dose of aspirin (3 mg/min; estimated to inhibit the production of PGI_2 by 95%) on forearm blood flow and resistance. Ten subjects with a mean age of 21.1 ± 2.5 yr (±SD) were recruited for this study. Forearm blood flow and blood pressure were measured after 5, 10, and 15 min of infusion of aspirin. In a subset of five subjects, measurements were performed every 10 min for a further 30 min to ensure that the effect was maintained. Forearm blood flow in the contralateral arm served as a time control for the effect of aspirin.

Dose-response relationship. Once the effect of aspirin on forearm blood flow and resistance was established, along with its time course of action, a second protocol was utilized to determine the dose-response relationship to aspirin. Three incremental doses of aspirin (1, 3, and 10 mg/min) or placebo (vehicle infusion) were infused into the forearm of 11 subjects [mean age 21.5 ± 3.4 yr (±SD)] to establish a cumulative dose-response effect. Each dose was infused for 10 min (based on the results of the first protocol) before forearm blood flow and blood pressure were determined. Approximately 1 min elapsed between each dose of aspirin. Forearm blood flow in both the control subjects (vehicle infusion) and the contralateral arm of the six subjects who received aspirin served as a time control for the effect of aspirin.

Arteriovenous sampling study. To be sure that any apparent changes in blood flow were due to inhibition of PGI_2 production and not the consequence of some indirect or secondary effect of aspirin, nine subjects [mean age 20.8 ± 1.6 (±SD)] underwent arteriovenous blood sampling for 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2), O_2 saturation, pH, norepinephrine, and 3,4-dihydroxyphenylglycol levels (an index of neuronal norepinephrine uptake) before and after the three doses of aspirin. Two of these subjects received two rather than three doses of aspirin. Venous blood was also taken after the three doses of aspirin for measurement of plasma salicylate levels. Forearm blood flow and blood pressure were again measured bilaterally at baseline and after each dose.

Vasodilator study. In a fourth protocol undertaken in eight subjects [mean age 20.4 ± 2.0 yr (±SD)], the cumulative effects of the three doses of aspirin on the vasodilator response to the endothelium-dependent vasodilator acetylcholine (30 μg/min) and to the endothelium-independent vasodilator sodium nitroprusside (1 μg/min) were studied. Forearm blood flow was measured bilaterally at baseline, after the three doses of aspirin, and during a 5-min infusion of each vasodilator before and after aspirin. Mean arterial blood pressure was measured at each forearm blood flow determination. The second determination of each vasodilator response was performed during coinfusion of aspirin and the respective vasodilators.

Hemodynamic measurements. Forearm blood flow was measured bilaterally by venous occlusion plethysmography (D. E. Hokanson, Bellevue, WA) and is expressed in milliliters per 100 milliliters of forearm tissue per minute (21). Flow was assessed for at least 2 min, and an average of a minimum of five measurements was used for analysis. Mean arterial blood pressure was measured from the intra-arterial catheter via a pressure transducer (Biosensors International, Singapore) at the end of each intervention. Forearm vascular resistance was calculated from mean arterial blood pressure and forearm blood flow.

Analog data were digitized on-line using an eight-channel analog-to-digital converter (MacLab/8s system, ADInstruments, Castle Hill, Australia) and were recorded directly to, and analyzed on, a multichannel chart recorder (Chart v. 3.5/s, ADInstruments) using a computer for data storage and subsequent analysis (Macintosh LC 630, Apple Computers, Cupertino, CA).

Biochemical analyses. Whole blood samples for 6-keto-$PGF_{1\alpha}$ were centrifuged and stored at −70°C until analysis (9). Quantification of 6-keto-$PGF_{1\alpha}$ was performed using a commercially available ^{125}I radioimmunoassay (DuPont, Wilmington, DE) and is expressed in picograms per milliliter (9, 15, 36). Extraction efficiency using this method is >90% (9), and our interassay variability was <6%.

Blood gas determination was performed using an ABL50 Blood Gas System (Radiometer, Copenhagen, Denmark). Plasma norepinephrine and 3,4-dihydroxyphenylglycol levels were measured using a well-validated high-performance liquid chromatography technique refined by Esler and coworkers (12) and are expressed in picograms per milliliter. Plasma salicylate (the stable metabolite of aspirin) levels were measured with a modified Trinder (44) colorimetric technique using the commercially available Dimension clinical chemistry system (DuPont) and are expressed in millimoles per liter.

Calculations. Venous and arterial 6-keto-$PGF_{1\alpha}$ content was calculated from the respective concentrations of 6-keto-$PGF_{1\alpha}$ multiplied by forearm blood flow and is expressed as picograms per 100 milliliters of forearm tissue per minute. Net forearm production of 6-keto-$PGF_{1\alpha}$, norepinephrine, and 3,4-dihydroxyphenylglycol was calculated by subtracting the arterial concentration from the venous concentration and multiplying by forearm blood flow (for 6-keto-$PGF_{1\alpha}$ and 3,4-dihydroxyphenylglycol) or forearm plasma flow (for norepinephrine). O_2 content and forearm O_2 consumption were calculated as previously described (16).

Statistical analysis. Baseline subject data are expressed as means ± SD. All physiological and biochemical data are expressed as means ± SE. Time-course data were assessed using repeated-measures analysis of variance (ANOVA). The effects of serial doses of aspirin on forearm blood flow, 6-keto-$PGF_{1\alpha}$, and other biochemical indexes were also assessed using repeated-measures ANOVA. When a statistical difference was detected using ANOVA, the Bonferroni multiple-comparison procedure was used to define differences between the results. For the time-course and biochemical data the analysis was also extended by orthogonal partitioning. Forearm blood flow responses to aspirin versus vehicle (control) infusion were compared with the use of two-way repeated-measures ANOVA using Bonferroni's method. Student's *t*-test was used for comparison of paired data (responses to acetylcholine and nitroprusside before and after aspirin). Statistical significance was accepted as $P < 0.05$.

RESULTS

A total of 38 subjects with a mean age of 21.3 ± 2.5 yr (±SD, range 18 to 28; 13 female, 25 male) were recruited for these studies. Their morphometric characteristics are shown in Table 1.

Time course of the effect of aspirin. Infusion of aspirin resulted in a time-dependent reduction in forearm blood flow with a corresponding increase in forearm vascular resistance. With the 3 mg/min dose, the reduction in forearm blood flow was maximal after 10 min. Forearm blood flow at baseline and after 5, 10, and 15 min of aspirin infusion was 2.7 ± 0.3, 2.3 ± 0.3, 2.0 ± 0.3, and 2.1 ± 0.3 ml·100 ml forearm tissue^{-1}·min^{-1}, respectively ($P < 0.001$; see Fig. 1). The percentage reduction in forearm blood flow relative to baseline for the three doses was 15, 26, and 22%, respectively. Post hoc analysis using orthogonal partitioning revealed that the effect was apparent after 5 min and maximal after 10 min, with no further reduction after 15 min. Forearm vascular resistance at baseline and for the three time points was 35.6 ± 5.1, 43.9 ± 6.9, 49.7 ± 7.8, and 48.7 ± 7.7 arbitrary units ($P < 0.001$; see Fig. 1). This corresponded to an increase in forearm vascular

Table 1. *Subject clinical characteristics*

	Protocol				
	1	*2*	*3*	*4*	Total
n	10	11	9	8	38
Age, yr	21.1 ± 2.5	21.5 ± 3.4	20.8 ± 1.6	20.0 ± 2.0	21.3 ± 2.5
Male:female	7:3	5:6	8:1	5:3	25:13
Body mass index, kg/m^2	21.8 ± 1.9	21.3 ± 2.6	22.8 ± 2.7	23.4 ± 2.3	22.2 ± 2.4
Waist:hip ratio	0.83 ± 0.1	0.80 ± 0.1	0.84 ± 0.1	0.84 ± 0.1	0.83 ± 0.1
Mean blood pressure, mmHg	80.0 ± 9.0	78.8 ± 6.6	79.5 ± 6.6	81.4 ± 5.2	79.8 ± 6.7
Total cholesterol, mmol/l	4.9 ± 0.9	4.5 ± 0.6	4.4 ± 0.4	4.7 ± 1.1	4.6 ± 0.8
LDL cholesterol, mmol/l	2.9 ± 0.5	2.7 ± 0.2	2.7 ± 0.4	2.9 ± 0.9	2.8 ± 0.5

Values are means ± SD; *n* is no. of subjects. LDL, low-density lipoprotein.

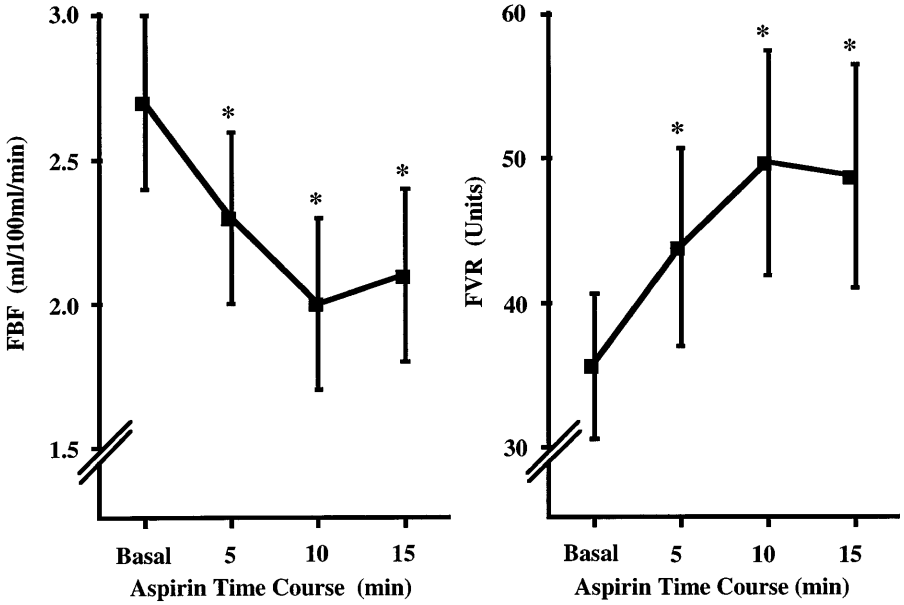

Fig. 1. Time-response curve for time course of action of aspirin (3 mg/min) on forearm blood flow (FBF) and forearm vascular resistance (FVR). FBF reduction occurred after 5 min and was maximal after 10 min of aspirin infusion, with a plateau thereafter [$*P <$ 0.001 for all 3 times compared with baseline (basal)]. Changes in FVR were concordant ($*P < 0.001$).

resistance of 23, 40, and 37% for the three time points, respectively. In contrast, there were no time-dependent changes in forearm blood flow in the subjects who received vehicle infusion and no time-dependent changes in flow in the contralateral arm of the subjects who received aspirin.

In the subset of five subjects in whom hemodynamic measurements were continued after maximal vasoconstriction was achieved, continuous infusion of the same dose of aspirin for a total of 45 min did not alter forearm blood flow, indicating a continuing effect of the drug.

Dose-response relationship. Data for a dose-response relationship using all three doses were available for 21 of the 28 subjects in the three remaining protocols. The three incremental doses of aspirin (1, 3, and 10 mg/min) decreased resting forearm blood flow by 8, 19, and 31%, respectively, compared with baseline. Forearm blood flow at baseline and for the three doses was 2.6 ± 0.2, 2.4 ± 0.2, 2.1 ± 0.2, and 1.8 ± 0.2 ml/100 ml forearm tissue$^{-1}\cdot$min^{-1}, respectively ($P < 0.001$; see Fig. 2). Forearm vascular resistance for baseline and the three respective doses was 33.7 ± 2.3, 39.0 ± 3.0, 45.9 ± 3.5, and 52.5 ± 4.6 units ($P < 0.001$, see Fig. 2). There was a corresponding increase in forearm vascular resistance compared with baseline of 16, 36, and 56%, respectively. Post hoc analysis revealed significant differences between each of the doses of aspirin for both forearm blood flow and vascular resistance.

Blood flow in the contralateral forearm of the subjects receiving the three doses of aspirin remained unchanged. Moreover, forearm blood flow during vehicle infusion in the five control subjects was unchanged: 2.9 ± 0.3, 2.8 ± 0.3, 2.9 ± 0.3, and 3.1 ± 0.2 ml$\cdot$100 ml forearm tissue$^{-1}\cdot$min^{-1} [P = not significant (NS); see Fig. 2]. When forearm blood flow for the control group was compared with that for the group

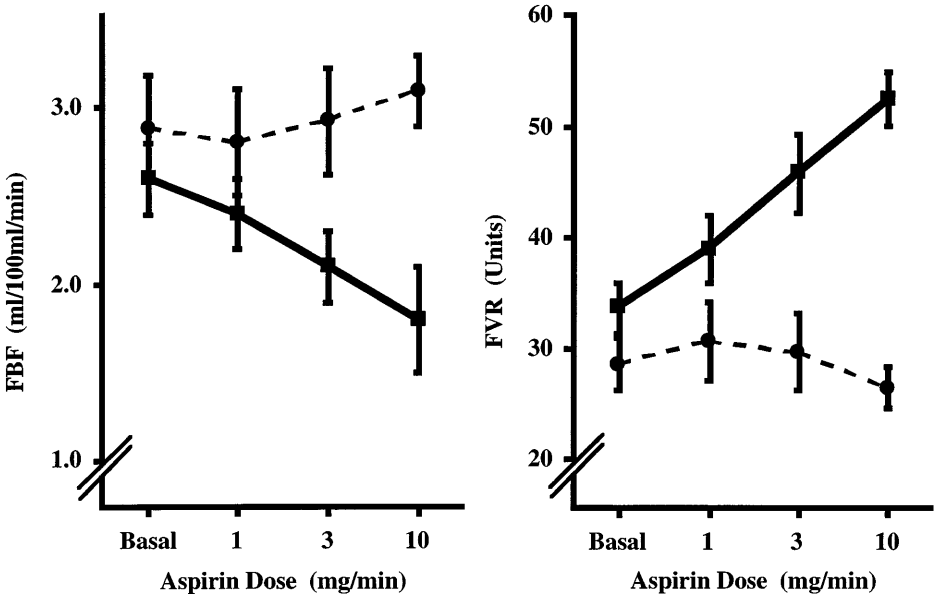

Fig. 2. Dose-response curve for dose- and time-response relationship of FBF and FVR to aspirin (1, 3, and 10 mg/min; ■; $n = 21$) and placebo (●; $n = 5$) infusions, respectively. FBF decreased by ~10% for each dose of aspirin ($P < 0.001$ for all 3 doses compared with baseline), and FVR increases were proportionate for each dose of aspirin ($P < 0.001$ for all 3 doses compared with baseline). FBF and FVR changes were different from those subjects who received vehicle infusion ($P < 0.001$).

that received aspirin, there was a significant difference between the groups ($P < 0.001$; see Fig. 2), indicating that the decrease in forearm blood flow seen with aspirin was not a time-related phenomenon. Mean arterial blood pressure did not change from baseline during the aspirin infusions [80.9 ± 1.3, 81.1 ± 1.6, 82.0 ± 1.7, and 82.1 ± 1.8 mmHg (P = NS)] and remained stable during the 45 min of continuous aspirin infusion.

Effect of aspirin on 6-keto-PGF$_{1\alpha}$ production. Forearm venous effluent 6-keto-PGF$_{1\alpha}$ concentration at baseline was higher than the arterial concentration. Venous levels were 54.8 ± 3.5 pg/ml, whereas arterial levels were 37.2 ± 4.9 pg/ml ($P < 0.01$), indicating net forearm production of 6-keto-PGF$_{1\alpha}$ at rest of 17.6 ± 4.5 pg/ml. Aspirin infusion produced a dose-dependent reduction in the venous content and net forearm production of 6-keto-PGF$_{1\alpha}$. Venous content of 6-keto-PGF$_{1\alpha}$ decreased by 25, 27, and 33% compared with baseline for the three respective doses of aspirin, with corresponding mean levels at baseline and the following three doses of 154.8 ± 23.6, 116.0 ± 28.9, 112.3 ± 23.5, and 103.1 ± 26.0 pg·100 ml forearm tissue^{-1}·min^{-1}, respectively ($P < 0.005$; see Fig. 3). Post hoc analysis using orthogonal partitioning revealed that there was a significant difference between the venous content after all three doses of aspirin compared with that at baseline ($P < 0.005$). There was a strong correlation between venous content of 6-keto-PGF$_{1\alpha}$ and forearm blood flow ($r^2 = 0.82$, $P < 0.001$; see Fig. 4).

In addition, net forearm production of 6-keto-PGF$_{1\alpha}$ declined by 78, 65, and 77% compared with baseline for the three doses of aspirin, with corresponding mean levels at baseline and the three doses of 52.9 ± 16.4, 11.7 ± 8.6, 18.7 ± 8.5, and 12.0 ± 12.5 pg·100 ml forearm tissue^{-1}·min^{-1}, respectively ($P = 0.04$; see Fig. 5). Post hoc analysis using orthogonal partitioning revealed that there was a significant difference between the net forearm content after all three doses of aspirin compared with baseline ($P < 0.005$), although there was no difference between the 1 and 10 mg/min doses, indicating a plateau of drug effect on net forearm production. Arterial content of 6-keto-PGF$_{1\alpha}$ was unchanged by the infusions of aspirin (data not shown). There was also a correlation between net forearm production of 6-keto-PGF$_{1\alpha}$ and forearm blood flow ($r^2 = 0.21$, $P < 0.01$; see Fig. 6).

Effect of aspirin on forearm O_2 consumption. Overall forearm O_2 consumption did not change. Consistent with the reduction in forearm blood flow produced by

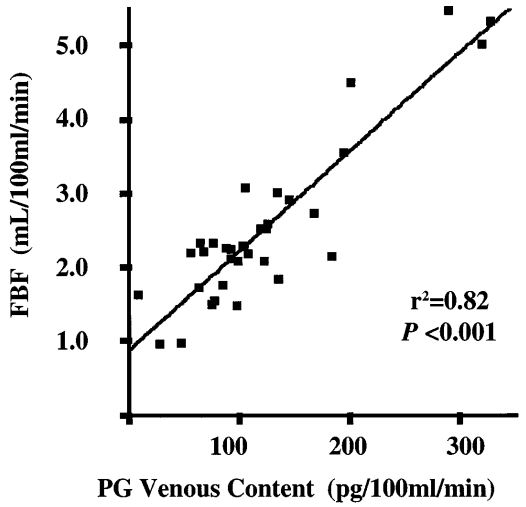

Fig. 4. Linear regression analysis for correlation of FBF to venous effluent content of PG. There is a strong correlation between venous content of PG (the stable metabolite of PGI_2) and FBF ($r^2 = 0.82$, $P < 0.001$).

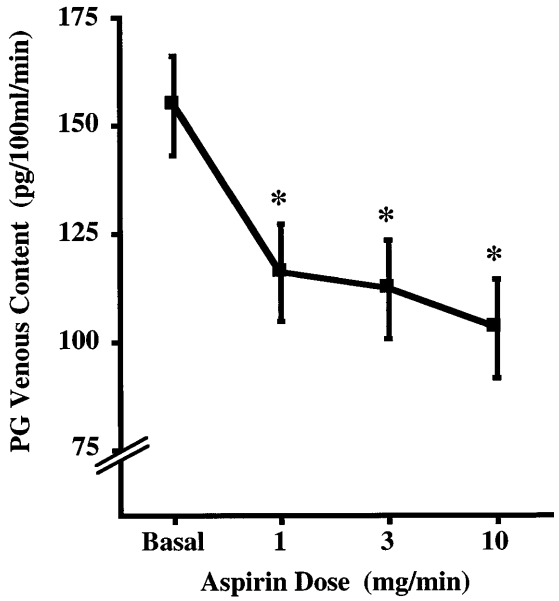

Fig. 3. Dose-response curve for effect of aspirin infusion on venous content of 6-keto prostaglandin F$_{1\alpha}$ (PG). Aspirin reduced the venous content of this stable metabolite of prostacyclin (PGI_2) in a dose-dependent fashion [*$P < 0.005$ vs. baseline by analysis of variance (ANOVA)].

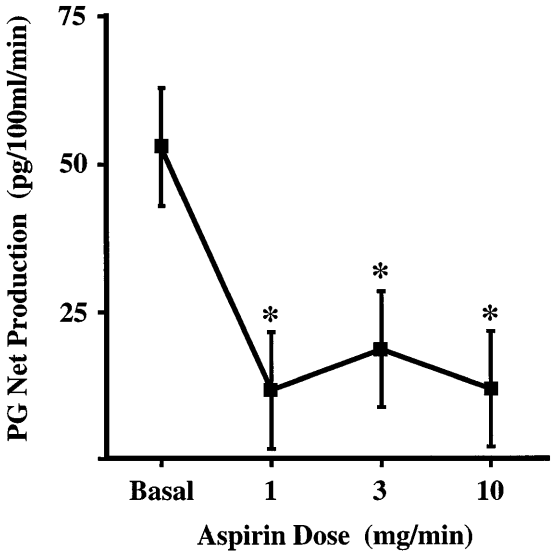

Fig. 5. Dose-response curve for effect of aspirin infusion on net forearm production of PG. Aspirin reduced net forearm production of this stable metabolite of PGI_2 ($P = 0.04$ by ANOVA), with a plateau effect after the 1 mg/min dose. Production after all 3 doses was different from baseline (*$P < 0.005$).

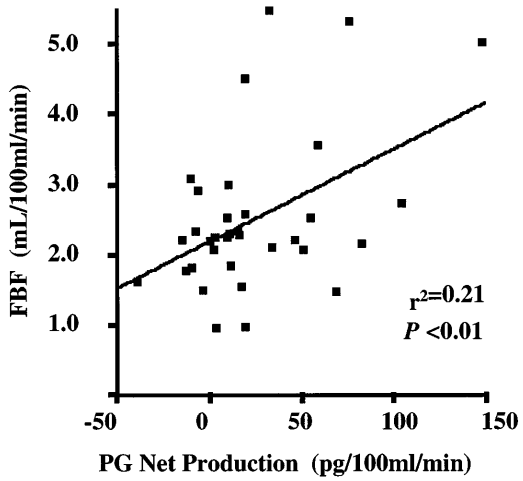

Fig. 6. Linear regression analysis for correlation of FBF to net forearm production of PG. There was a correlation between net forearm production of the stable metabolite of PGI_2 and FBF ($r^2 = 0.21$, $P < 0.01$).

the infusion of aspirin, there was a modest increase in forearm O_2 extraction (30% for the highest dose compared with baseline), although this did not reach statistical significance. Notably, there was no significant change in arterial or venous pH or bicarbonate.

Effect of aspirin on forearm norepinephrine production. Arterial and venous norepinephrine and 3,4-dihydroxyphenylglycol data at baseline and after all three doses of aspirin were available for seven subjects (*protocol 3*). At baseline, arterial and venous norepinephrine and 3,4-dihydroxyphenylglycol concentrations were similar. Aspirin infusion produced no discernible alteration in the net forearm production of norepinephrine or 3,4-dihydroxyphenylglycol.

Effect of aspirin on salicylate levels. Because the plasma half-life of acetyl salicylic acid is ~15 min (22, 39), the forearm venous effluent concentration of salicylate (the stable metabolite of aspirin) approximates systemic levels rather than reflecting the local concentration of acetyl salicylic acid. Infusion of aspirin resulted in a dose-dependent increase in the venous concentration of salicylate, with levels for the three respective doses of aspirin of 0.1 ± 0.0, 0.2 ± 0.0, and 0.5 ± 0.1 mmol/l ($P < 0.001$). Post hoc analysis revealed a significant difference between each mean level (baseline levels were not taken for comparison). These salicylate levels correspond to the peak levels found 2 h after oral administration of 325 mg, 650 mg, and 1.5 g of commercially available aspirin (28) and are substantially below the levels required for a therapeutic anti-inflammatory effect (150–300 μg/ml) (22).

Effect of aspirin on responses to acetylcholine and nitroprusside. The forearm blood flow responses to endothelium-dependent and -independent vasodilation were not affected by the infusion of aspirin. The forearm blood flow with acetylcholine before and after aspirin was 16.7 ± 3.2 and 20.2 ± 3.3 ml·100 ml forearm tissue^{-1}·min^{-1}, respectively (P = NS). The forearm blood flow with nitroprusside before and after aspirin was 5.7 ± 0.5 and 6.1 ± 0.8 ml·100 ml forearm tissue^{-1}·min^{-1}, respectively (P = NS).

DISCUSSION

Previous investigations of the role of PGI_2 in the control of blood flow in humans have indicated that PGI_2 is principally involved in hyperemia secondary to ischemia and exercise, with little or no role in the maintenance of resting flow (4, 11, 25). In this study we have demonstrated that infusion of the cyclooxygenase inhibitor aspirin directly into the brachial artery results in a dose-dependent reduction in resting forearm blood flow in healthy humans. This reduction in flow was associated with diminished venous effluent content and net forearm production of the stable metabolite of PGI_2, namely 6-keto-$PGF_{1\alpha}$, suggesting that decreased production of PGI_2 contributed to the reduction of blood flow.

Apart from the dose-dependent reduction in forearm blood flow, there was also a time-dependent decrease in flow with a corresponding increase in forearm vascular resistance. The observed time course of the effect of aspirin is consistent with its known pharmacokinetic profile. Jaffe and Weksler (23) demonstrated a half-time of 6 min for the inhibition of cyclooxygenase with aspirin in cultured human endothelial cells. Using a single-dose infusion of aspirin, we observed an effect on resting hemodynamics after 5 min, with a maximal effect after 10 min and no further change after 15 min. A continued effect of aspirin was observed for at least 45 min, consistent with continuous inhibition of cyclooxygenase and, thus, PGI_2 production. This is concordant with findings in cultured endothelial cells in which the inhibition of cyclooxygenase with aspirin may take 36 h to recover (23).

In our dose-response experiments we found that each dose increase resulted in an ~10% further reduction in forearm blood flow, with a maximal mean reduction in flow of 31% with the 10 mg/min dose. There was a corresponding maximal increase in forearm vascular resistance of 56% with this dose. These changes in resting forearm hemodynamics are comparable with those seen in animal studies of cyclooxygenase inhibition in skeletal muscle vasculature (3, 24, 50). Moreover, these changes occurred despite a lack of effect on mean arterial blood pressure and contralateral forearm blood flow.

To ascertain whether the changes in resting hemodynamics were a result of inhibition of PGI_2 production or some other pharmacological or nonspecific effect, we measured the forearm production of the stable metabolite of PGI_2, 6-keto-$PGF_{1\alpha}$, in response to aspirin infusion and found a reduction of both the venous effluent content and the net forearm production of 6-keto-$PGF_{1\alpha}$. Interestingly, although we observed a progressive decrease in forearm blood flow with increasing doses of aspirin, the reduction in net production of 6-keto-$PGF_{1\alpha}$ appeared to plateau after the 1 mg/min dose of aspirin. This observation is consistent with the findings of Masotti and colleagues (29), on whose findings we based our aspirin dose calculations. Their

bioassay data suggested that the 1 mg/min dose would inhibit PGI_2 production by ~80%, with only modest added inhibition with higher doses (29). Wilson and Kapoor (49) noted that indomethacin decreased both 6-keto-$PGF_{1\alpha}$ and prostaglandin E_2 (PGE_2) release in the forearm of humans. The further reduction in forearm blood flow in our study with the 3 and 10 mg/min doses of aspirin occurred while net forearm production of 6-keto-$PGF_{1\alpha}$ was similar. This could be due to reduction of PGE_2 release; however, aspirin is unlikely to have a differential effect on these two prostanoids.

The reduction we observed in venous effluent 6-keto-$PGF_{1\alpha}$ content in response to increasing doses of aspirin was, however, more linear. Despite the disparity between the two dose-response curves (forearm blood flow vs. aspirin dose and 6-keto-$PGF_{1\alpha}$ vs. aspirin dose), we observed a strong correlation between the reduction in the venous content of 6-keto-$PGF_{1\alpha}$ and forearm blood flow and a significant correlation between net forearm production of 6-keto-$PGF_{1\alpha}$ and forearm blood flow, suggesting that the reduction in forearm blood flow was due to inhibition of PGI_2 production.

Mechanisms. The most likely mechanism by which aspirin exerted its effect is through the progressive reduction of the production of the vasodilator PGI_2. Another possible mechanism might be that aspirin inhibited other prostaglandins such as PGE_2. This prostaglandin has been implicated in the control of resting and stimulated skeletal muscle blood flow in experimental models (19, 50, 51) and in humans (25, 33, 49). However, PGI_2 is the principal vascular prostanoid produced in human forearm vasculature (33).

There are several other possible mechanisms by which aspirin may have produced its effect, including shifting the balance in favor of vasoconstrictors. As a result of the inhibition of cyclooxygenase, aspirin may have resulted in the preferential metabolism of arachidonic acid via the lipooxygenase pathway with the production of leukotrienes (6). Leukotrienes have been shown to be vasoconstrictors in a number of vascular beds in several species (35). Although we did not measure leukotrienes in this study, this effect of aspirin has only been demonstrated to be important in bronchial smooth muscle cells (41). A second possible mechanism may have been by a direct vasoconstrictor effect of aspirin on vascular smooth muscle when given in the anti-inflammatory dose range. This seems unlikely because the time course of the effect of aspirin on blood flow suggests that the drug is affecting a biosynthetic pathway, which, as was alluded to earlier, is consistent with the known in vitro time course of inhibition of cyclooxygenase by aspirin in human endothelial cells (23).

Although aspirin is a weak acid (pK_a 3.5), we found no evidence to indicate that the fall in forearm blood flow during aspirin infusion could be explained by an effect on plasma pH. Moreover, other investigators (43) who used a similarly weak acid, ascorbic acid, have not demonstrated any effect on resting forearm hemodynamics.

Another possible mechanism might be that aspirin induced vasoconstriction indirectly through either central or local modulation of sympathetic efferent outflow. The former is unlikely in view of the fact that local aspirin infusion was not associated with a rise in arterial plasma norepinephrine levels and did not result in a rise in systemic arterial pressure. A local effect on norepinephrine release or reuptake is possible. Inhibition of cyclooxygenase has previously been demonstrated to potentiate the vasoconstrictor effect of norepinephrine in experimental models (31). Moreover, prostanoids, among other endogenous compounds such as acetylcholine, can inhibit the release of norepinephrine from sympathetic nerve endings (47). With this in mind, we measured the arteriovenous production of norepinephrine and 3,4-dihydroxyphenylglycol levels (an index of neuronal norepinephrine uptake) and found no evidence of increased local release or altered uptake of norepinephrine during aspirin infusion.

Endothelium-derived vasodilators. The endothelium produces a number of vasoactive substances including nitric oxide and PGI_2 (27, 46). Although similar factors have been proposed as endogenous stimuli for the release of nitric oxide and PGI_2, such as activated platelets and bradykinin (18), and the receptor-mediated release of both substances may be linked (10), nitric oxide and PGI_2 are thought to play different roles in the regulation of skeletal muscle blood flow (17). Vallance and colleagues (45) elegantly demonstrated the tonic release of nitric oxide in the human forearm by intra-arterial infusion of the competitive inhibitor of nitric oxide production, N^G-monomethyl-L-arginine, which reduced resting blood flow by 50%. In this investigation we have shown that a comparable decrease in forearm blood flow can be achieved with intra-arterial aspirin.

Acetylcholine causes vasodilation in skeletal muscle vasculature, largely due to receptor-mediated release of nitric oxide (45). In the present study we found that aspirin did not alter acetylcholine-induced vasodilation, suggesting, although not proving, that receptor-mediated nitric oxide-dependent vasodilation was not altered by aspirin infusion. Similarly, the vasodilator responses to sodium nitroprusside before and after aspirin infusion were not altered, indicating that nitric oxide-linked guanosine 3′,5′-cyclic monophosphate-mediated vasodilation was preserved.

Evidence from experimental and previous human studies. Our findings are consistent with several animal studies that have demonstrated that maintenance of resting skeletal muscle blood flow is at least in part dependent on the continuous release of prostaglandins (3, 24, 50). Resting blood flow was reduced and vascular resistance was increased by ~40% in these studies. In addition, vasodilator prostanoids have been implicated in the control of resting blood flow in other circulatory beds, including the coronary circulation (1, 2, 20).

In the human coronary circulation, Friedman et al. (14) investigated the effect of intravenous indomethacin in patients with severe coronary artery disease and found that it increased blood pressure, coronary vascu-

lar resistance, and myocardial O_2 extraction, whereas it decreased coronary blood flow. However, an investigation into the effects of cyclooxygenase inhibitors in skeletal muscle vasculature by Kilbom and Wennmalm (25) did not identify any effect of prostaglandins on resting blood flow. They found that administration of indomethacin reduced total functional hyperemia by 42% and total reactive hyperemia by 48% (25). Nevertheless, while investigating the role of prostaglandins in exercise-induced vasodilation in humans with the use of intra-arterial indomethacin, Wilson and Kapoor (49) noted a 23% decrease in resting forearm blood flow, with a significant decrease in 6-keto-$PGF_{1\alpha}$ and PGE_2 release. We are unaware of other studies in which aspirin has been delivered intra-arterially in humans.

Several studies of the estimated rate of PGI_2 production have suggested that PGI_2 is unlikely to be produced in the resting state in healthy humans (13, 38). This conclusion is based on the evidence that basal plasma PGI_2 levels are usually in the picogram-per-milliliter range and that substantially higher concentrations of exogenous PGI_2 ($ng \cdot ml^{-1} \cdot min^{-1}$) are required to achieve vasodilation in vivo (34). However, circulating plasma levels of PGI_2 or its metabolite, 6-keto-$PGF_{1\alpha}$, may not reflect the local vascular biological activity of PGI_2. PGI_2 is not a circulating hormone (5), and its paracrine actions probably occur on platelets and vascular smooth muscle cells immediately adjacent to endothelial cells in healthy vessels (18, 34).

Clinical implications. Our findings and those of Friedman and colleagues (14) indicate that it would be prudent to exercise caution when the use of cyclooxygenase inhibitors in the anti-inflammatory dose range is being considered in patients with peripheral or coronary atherosclerosis.

Although the aspirin levels achieved were estimated to be in the anti-inflammatory range (22, 28, 39, 48), lower doses may have important hemodynamic effects. In the Study on Left Ventricular Dysfunction (SOLVD), cardioprotective doses of aspirin negated the benefit that enalapril had on prognosis of patients with heart failure (7). It is possible that this reflected a cumulative effect of aspirin. Angiotensin-converting enzyme inhibitors achieve part of their beneficial vasodilating effect by increasing bradykinin, which is known to stimulate the release of both PGI_2 and nitric oxide (18). Both aspirin and indomethacin have been shown to attenuate the beneficial hemodynamic effects of angiotensin-converting enzyme inhibitors in humans (7). Although we have not studied patients with heart failure, our data suggest a further mechanism to explain how cyclooxygenase inhibitors may adversely affect the peripheral arterial tone in patients with heart failure.

Study limitations. It is possible that the insertion of the brachial artery line itself stimulated the release of vasodilators such as PGI_2 and nitric oxide and that inhibition of prostanoid production with aspirin merely returned blood flow back to normal. To overcome this problem, we waited at least 30 min after line insertion before taking the first measurement to allow any effects of the procedure to resolve (30, 45). Moreover, in 13 of our aspirin studies, we measured resting flow before and after arterial line insertion and found no difference (2.2 ± 0.2 before vs. 2.6 ± 0.3 ml·100 ml forearm $tissue^{-1} \cdot min^{-1}$ after line insertion, P = NS).

The reproducible dose and time responses to aspirin indicate that it was the concentration of aspirin that determined the inhibition of vasodilator prostanoids, but it is possible that the reduction in blood flow was due to a cumulative effect of aspirin. The near-constant forearm blood flow during prolonged infusion of aspirin and the plateau in response to a single dose of aspirin after 10 min suggest that the response is dose related, however.

Although we have excluded an effect of aspirin on norepinephrine production, we did not assess whether aspirin affected the vasoconstrictors angiotensin II or endothelin. The interaction between vascular prostanoids and these vasoconstrictors in humans warrants further investigation.

In conclusion, this study has shown that infusion of the cyclooxygenase inhibitor aspirin into the forearm of resting healthy humans results in a dose-dependent reduction in forearm blood flow and that this correlates with diminished production of 6-keto-$PGF_{1\alpha}$, the stable metabolite of PGI_2. This indicates that PGI_2 contributes to the maintenance of resting blood flow to skeletal muscle in humans and may have important implications for the use of cyclooxygenase inhibitors in patients with some cardiovascular diseases.

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