Repeated Sympathetic Stimuli Elicit the Decline and Disappearance of Prostaglandin Modulation and an Increase of Vascular Resistance in Humans

Gian Gastone Neri Serneri, Sergio Castellani, Luca Scarti, Francesco Trotta, Ji Lin Chen, Marino Caronvali, Loredana Poggesi, and Giulio Masotti

To investigate the role of prostaglandins I$_2$ and E$_2$ in modulating the vasoconstrictor response to sympathetic stimulation, repeated and proximate cold pressor tests were performed in 23 healthy young volunteers. Limb vascular resistance (blood flow measured by venous occlusion plethysmography), prostaglandin I$_2$ (as 6-ketoprostaglandin F$_1\alpha$) and prostaglandin E$_2$ plasma levels (detected by radioimmunoassay), and plasma catecholamines (detected by high-performance liquid chromatography and electrochemical detection) were measured. A progressive increase in the duration of the vasoconstrictor response was observed with repetition of cold applications ($p<0.001$, by analysis of variance for trends). This increase was associated with a progressive decrease in cold-induced elevation of 6-ketoprostaglandin F$_1\alpha$ and prostaglandin E$_2$ plasma levels until, after five stimulations, neither prostaglandin was detectable. The maximum detected concentration of norepinephrine did not significantly change, but its area under the curve in time showed a trend toward an increase. Epinephrine levels did not significantly change. The increase of vascular resistance was significantly correlated with the decrease of both prostaglandins ($r=0.93$, $p<0.05$ for prostaglandin E$_2$ and $r=0.89$, $p<0.05$ for 6-ketoprostaglandin F$_1\alpha$), whereas no significant correlations were found between variations of vascular resistance and catecholamines. Prostaglandin blockade induced by diclofenac sodium administration caused, from the first cold application, a pattern of the vasoconstrictor response and plasma prostaglandin and norepinephrine changes similar to that observed at the fifth cold application in untreated subjects, when prostaglandins are no longer detectable in plasma. We conclude that an increased vasoconstrictor response to sympathetic stimulation in humans may result from a diminished inhibitory influence of prostaglandins on adrenergic transmission. (Circulation Research 1990;67:580–588)

In animal experiments, both exogenous catecholamines and nerve stimulation have been reported to induce prostaglandin release from different organs such as the spleen$^3$ and the kidneys$^2$ as well as from isolated blood vessels.$^3$–$^6$ In tissue fragments and isolated perfused organs, prostaglandin I$_2$ (PGI$_2$) and prostaglandin E$_2$ (PGE$_2$) have been found to modulate the effects of sympathetic stim-

ulation.$^6$–$^{12}$ However, the effects of prostaglandins on adrenergic neurotransmission and vascular reactivity to adrenergic stimulation are species dependent and may also vary in different vascular beds within the same species.$^6$ In humans the role of prostaglandins in modulating vascular response to sympathetic stimulation is still not well defined. Sympathetic stimulation induced by cold application in healthy volunteers caused significant increases in PGI$_2$ and PGE$_2$ levels in plasma.$^{13,14}$ Moreover, an increased transcardiac gradient of 6-ketoprostaglandin F$_1\alpha$ (6-keto-PGF$_{1\alpha}$) and PGE$_2$ has been found in nonischemic subjects after sympathetic stimulation.$^{15}$ The aim of the present study was to further investigate the role of endogenous prostaglandins in modulating the vasoconstrictor response to sympathetic nerve activation. It has been previously demonstrated both in vitro$^{16}$ and locally in humans$^{17}$ that prostaglandin release

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can be exhausted by the repetition of various stimuli active on prostaglandin synthesis. We investigated whether repeated sympathetic stimulation induced by cold application could also result in an exhaustion of prostaglandin response and, if so, whether such exhaustion modified the vascular response to sympathetic activation.

Subjects and Methods

Subjects

Studies were carried out on 23 healthy volunteers, who had given their informed consent to participate in the study. They were nonsmokers (14 males and nine females), aged 22–32.

Subjects were considered healthy on the basis of their history, a physical examination, an ECG, and a chest x-ray. Offspring of hypertensive parents were not included in the study because in these subjects vascular response to sympathetic stimulation can be altered.18 The subjects were studied 3 hours after a light breakfast without lipids or milk. No subject had taken aspirin or other cyclooxygenase inhibiting drugs for 10 days. All investigations were performed in a quiet room with a constant temperature (22°C). The subjects lay in a supine position for at least 1 hour before and during the study.

Sympathetic Stimulation

Sympathetic stimulation was induced as already described13,14 by immersing one foot in a pan containing equal parts of water and ice (temperature 0–2°C), and by simultaneous application of ice to the laterocervical regions for 120 seconds. Care was taken to avoid compression of the carotid sinuses.

The five cold stimulations were applied one after the other as soon as the calculations showed that the peripheral vascular resistance (PVR) had returned to baseline. This return took from 6 to 10 minutes.

Experimental procedure permitted variations of PVR to be recorded simultaneously with blood sampling for prostaglandins and catecholamines. Therefore, blood samples were drawn from a brachial vein, blood pressure was measured in the other arm, and blood flow was measured in a leg. In fact, the response to the cold pressor test is known to be a systemic reaction.19 In a preliminary study, we found that it elicits similar hemodynamic effects in the arm and calf of the leg. In this preliminary study of five healthy subjects, baseline arterial blood flow was found to have different values in the arm and calf (3.03 ± 0.47 and 2.07 ± 0.65 ml/100 ml/min, respectively), but the reduction in response to the cold pressor test was not statistically different (−35.2 ± 17.3% and −34 ± 16.6%, respectively). Therefore, the cold-induced increase in PVR had a similar pattern in the arm and calf. Thus, the increase in peak PVR was +81.7 ± 21% in the forearm and +89.4 ± 17.6% in the calf (p > 0.05 by covariance analysis). The area under the curve with respect to time for PVR was 54.3 ± 10.4 and 58.2 ± 8.4 units/min for forearm and calf, respectively (p > 0.05, Student’s t test). Units of PVR were obtained by dividing mean blood pressure by calf blood flow. The time for return of PVR to baseline value was 1 minute after cessation of cold in both the arm and calf.

A 16-gauge Teflon catheter needle with a three-way stopcock was inserted in the antecubital vein to collect blood samples for prostaglandin and catecholamine assays. To prevent clotting, a glucose isotonic solution was continuously infused at a low flow rate (0.2 ml/min) through the catheter, which was flushed with about 2–3 ml after each blood sampling. Blood samples for basal values were obtained at least 15 minutes after venipuncture and at a time when the subjects appeared to be relaxed. For each cold application, blood samples for prostaglandin and catecholamine assays were taken before (100 seconds from the beginning) and then at 4, 8, and (when necessary) 12 minutes after the beginning of the cold application and immediately before applying the subsequent cold application. During the entire experiment, the total blood sampling for prostaglandins and catecholamines amounted to about 70 ml for study A and to about 90 ml for study B (see “Experimental Protocol”). During the experiment, these volumes were practically replaced by the continuous infusion of glucose solution used to prevent catheter clotting.

Lower limb vascular resistance was measured every 30 seconds during cold application and every minute thereafter. Before the first cold application, four measurements were made, and the average was taken as the baseline value.

Prostaglandin Assay

Blood samples (1.5 ml) for the assay were withdrawn without stasis directly into cold polypropylene syringes containing 0.15 ml of a 100 μg/ml fenoprofen solution in 0.037 M EDTA to avoid the formation of cyclooxygenase-mediated arachidonic acid metabolites by platelets during the handling of the samples.20,21 The samples were centrifuged at 3,800g for 30 minutes at 4°C within 1 hour of collection, and the separated plasma was stored at −20°C until the time of assay. Plasma PGE2 was measured by radioimmunoassay according to Patrono et al.22 The detection limit was 5 pg/ml. Values for intra-assay and interassay coefficients of variation were 9% and 10%, respectively. A more detailed description of the method has been extensively published elsewhere.14

The prostacyclin plasma concentration has been evaluated by measuring its stable metabolite 6-keto-PGF1α by radioimmunoassay in unextracted, platelet-poor plasma according to the method of Patrono et al.23 with the one exception that only 0.04 ml plasma was used to prevent nonspecific interferences with the antigen-antibody binding. Duplicate standard curves were prepared in a total volume of 0.25 ml/tube, consisting of standard 6-keto-PGF1α in 0.21 ml of 0.02 M phosphate buffer (pH 7.4) and 0.04 ml stripped plasma (charcoal-treated prostaglandin-free
plasma). A 1.25-ml incubation mixture was then added to each tube containing 4,500 dpm of tritium-labeled 6-keto-PGF1α (120–170 Ci/mmol; NEN, Dreieich, FRG) and the 1:100,000 diluted antibody solution in 0.02 M phosphate buffer (pH 7.4).

After 16–18 hours of incubation at 4°C, 35–45% binding was obtained. The separation of free 6-keto-PGF1α from antibody-bound 6-keto-PGF1α was obtained by the addition of 10 mg uncoated charcoal (Norit A, Serva, Feinbiochemia, Heidelberg, FRG) and subsequent centrifugation at the same temperature. The antiserum raised in the rabbit was kindly provided by Dr. Bernard D. Peskar (Department of Pharmacology, Bochum University, Bochum, FRG). The detection limit of the method was 5 pg/ml. Values for intra-assay and interassay coefficients of variation were 5.5% and 9.6%, respectively. The maximum cross-reactivity for prostaglandins and related substances was 1.9% with 6-keto-PGE1 and less than 0.3% for the others.

**Cathecolamine Assay**

Cathecolamines were determined according to the method described by Mefford et al. and were based on separation by high-pressure liquid chromatography and electrochemical detection, which permits simultaneous determination of norepinephrine and epinephrine. Blood samples (2 ml) were drawn into cold heparinized tubes; after centrifugation, the plasma was frozen and stored at −70°C until analysis.

After the addition of 3,4-dihydroxybenzylamine as an internal standard, cathecolamines were adsorbed onto acid-washed aluminum oxide. Perchloric acid was used to remove them from the acid-washed aluminum oxide. This was then injected into a high-performance liquid chromatography separation system (model 6000 A chromatographic pump, Waters Chromatography Division, Millipore Corp., Milford, Mass.; 6017 ODS Sum Biophas column, Bioanalytical System, Inc., West Lafayette, Ind.), and the effluent was passed over a detector (LC4B Amperometric Detector, Bioanalytical). Chromatograms were recorded on a D-5000 Omniscribe strip chart recorder (Gistel, Belgium).

**Measurement of Limb Vascular Resistance**

Limb vascular resistance was calculated by dividing the mean blood pressure by lower limb blood flow and was expressed in units (see “Sympathetic Stimulation”). Arm blood pressure was measured with a mercury sphygmomanometer; the first Korotkoff sound was taken as an index of systolic pressure and the fifth as an index of diastolic pressure. Mean arterial pressure was obtained by adding one third of the pulse pressure to the value of the diastolic. Lower limb blood flow was measured by venous occlusion strain gauge plethysmography (SU 4 Periflow 4, Janssen Scientific Instruments, Beersel, Belgium) and was expressed in milliliters per 100 ml per minute. Each value was obtained from the average of five consecutive measurements. Each measurement was automatically made by the plethysmograph, which, synchronized with the heart rate, occluded the veins for three heart beats and released them for two.

**Experimental Protocol**

The study was performed in two successive phases. In phase A, 15 subjects underwent five successive cold stimulations. The aim was to evaluate the effects of repeated sympathetic stimulations on prostaglandins, cathecolamines, and vascular resistance. Phase B was designed to study the effects of repeated sympathetic stimulations after pharmacological inhibition of prostaglandin synthesis. In this phase, eight other subjects were submitted twice to five successive cold stimulations: one of them took place after a 3-day treatment with placebo and the other after a 3-day treatment with diclofenac sodium (200 mg/day p.o.). The two treatments were administered in a randomized sequence and were separated by a 10-day washout period. Diclofenac was used as a cyclooxygenase inhibitor because it has been proved to be a very effective inhibitor of prostaglandin synthesis and devoid of direct vasoconstrictor activity.

The modification induced by each cold stimulation on prostaglandin formation, cathecolamine release, and PVR has been studied by evaluating 1) variations of the maximum detected plasma concentration of prostaglandins and cathecolamines and of the peak value of PVR, 2) variations of the area under the curve with respect to time for prostaglandin and cathecolamine plasma concentrations and for PVR, and 3) the time intervals at which the cathecolamine plasma concentration and the value of the PVR (after the cold-induced elevation) were no longer significantly different from their precold values.

The areas under the curve with respect to time were calculated by the trapezoid method and were expressed as picograms per milliliter per minute (for prostaglandins and cathecolamines) or units per minute (for PVR).

**Statistical Analyses**

Statistical significance of the differences in the responses induced by cold stimulation was evaluated by analysis of variance (ANOVA) and Tukey's test. Whether or not the changes in PVR, prostaglandin production, and cathecolamine release were dependent on the repetition of cold applications, they were evaluated by ANOVA for trends according to Scheffe. A linear regression analysis was performed to test the correlation between PVR and cathecolamines, PVR and prostaglandins, and cathecolamines and prostaglandins, all expressed as areas. The one-way and two-way ANOVAs were used for comparison of the effects of sympathetic stimulation with placebo versus diclofenac treatment (phase B experiments) and versus those observed in untreated subjects (phase A), respectively.
Results

The first cold application induced a significant increase in PVR (p<0.001 by ANOVA for the whole curve) associated with a marked increase in plasma levels of norepinephrine (p<0.001), epinephrine (p<0.001), and prostaglandins (PGE₂, p<0.001; 6-keto-PGF₁α, p<0.001) (Figure 1).

The application of successive sympathetic stimulations significantly affected PVR and prostaglandin levels in venous blood. Indeed, subsequent cold applications resulted in an increase of the area under the curve with respect to time for PVR, and the largest area occurred at the fifth cold application (Figure 2, Table 1). Actually, the maximum increase in PVR was achieved in 10 subjects after five cold applications and in five subjects after four cold applications. This increase was progressive in time (ANOVA for trends p<0.001), and the difference in comparison to the first cold stimulation was significant (by Tukey’s test) by the fourth and fifth (p<0.05 and p<0.01, respectively; Table 1). The increase of the area under the curve for PVR was mainly due to the prolonged duration of the vasoconstrictor response, because the peak values of PVR did not significantly change with repetition of cold application (ANOVA). The duration of vasoconstrictor responses induced by cold application was significantly longer (Tukey’s test) by the third, fourth, and fifth stimulation in comparison to the first two. Indeed, in the first two cold applications, the return of PVR to the prestimulation values occurred at the third minute after the beginning of sympathetic stimulation, at the fourth minute with the third cold application, at the sixth minute with the fourth cold application, and at the seventh minute with the fifth stimulation (Figure 2). The progressive increase in vasoconstrictor response resulting from successive, sympathetic stimulations was associated with a significant decrease in prostaglandin levels in venous blood until both PGE₂ and 6-keto-PGF₁α were no longer detectable (Figure 3). The progressive reduction of plasma PGE₂ and 6-keto-PGF₁α levels was linear with the repetition of cold stimulations both for maximum detected concentrations (p<0.001, ANOVA for trends, for PGE₂ and 6-keto-PGF₁α) and areas (p<0.001 for PGE₂ and 6-keto-PGF₁α). In 13 of 15 subjects, the levels of 6-keto-PGF₁α became undetectable by the fourth stimulation, whereas for PGE₂ it occurred by the fifth (Figure 3). The decrease of 6-keto-PGF₁α after repetitive sympathetic stimulation reached statistical significance from the third stimulation onward (p<0.001 for maximum detected concentrations and p<0.001 for areas) and from the fourth onward for PGE₂ (p<0.001 for maximum detected concentrations and p<0.001 for areas; Figure 3, Table 2). The decrease of areas of both prostaglandins was inversely correlated with the increase in areas of PVR (r=0.93, p<0.05 for PGE₂; r=0.89, p<0.05 for 6-keto-PGF₁α) (Figure 4).

The maximum detected concentrations of plasma norepinephrine after each cold stimulation were not significantly different from each other (Figure 5), whereas the area under the curve changed after the repetition of cold stimulation (p<0.05 by ANOVA) with a trend toward an increase. However, only the area after the fifth stimulation was significantly greater than the first (p<0.05) (Table 1). The changes in the area under the curve for norepinephrine plasma concentration after the repeated sympathetic stimulations were not significantly related to the progressive increase in the area of PVR (r=0.84, p>0.05), whereas they were inversely related to the progressive decrease in the area of PGE₂ (r=0.92, p<0.05) but not of 6-keto-PGF₁α. Successive cold stimulations did not induce any
significant changes in epinephrine values (neither in maximum detected concentrations nor in areas) (Figure 5).

For the eight subjects studied in phase B, the changes induced by repeated cold stimulations on vascular resistance, plasma catecholamines, and prostaglandins during placebo treatment were not statistically different from those observed for the 15 subjects investigated in phase A. After prostaglandin inhibition induced by diclofenac administration, PVR, from the first cold application, was markedly altered. At the first cold application, even if the peak was not significantly enhanced, the area was instead significantly greater than during placebo treatment (178.5±54.2 versus 73.7±21.7 units/min, p<0.001, one-way ANOVA; Figure 6). Prostaglandin plasma concentrations were undetectable. Epinephrine expressed as area under the curve was equivalent to that after placebo treatment, and norepinephrine as area under the curve was higher than that during placebo treatment, but the difference did not reach statistical significance (1,181±268 versus 902±242 pg/ml/min, f=1.46, NS) (Table 3).

The subsequent cold application did not induce significant changes in the vasoconstrictor response, which remained similarly elevated. The area under the curve for PVR observed at the first cold stimulation after taking diclofenac was not significantly different from that observed after the fourth and the fifth stimulations, when subjects were on placebo (178.5±54.2 units/min versus 142.4±42.3 units/min, f=1.78, NS, and 169.9±50.4 units/min, f=0.62, NS, respectively) (Figure 6).

Norepinephrine plasma concentration (expressed as area under the curve) after diclofenac treatment was significantly greater than that of the first cold stimulation at the third through the fourth and fifth stimulations (p<0.05 for each stimulation). The area under the curve at the third cold stimulation in patients taking diclofenac was significantly greater than that observed at the third cold stimulation with placebo treatment (1,623±480 versus 917±257 pg/ml/min, p<0.05) (Table 3). No significant variations could be observed in the maximum detected concentrations.

Epinephrine plasma concentration during diclofenac treatment did not significantly change, when compared with the first stimulation, during the subsequent cold applications.

### Discussion

Present results indicate that repeated and proximate sympathetic stimulation results in a progressive increase in the vasoconstrictor response associated with a progressively lower or absent elevation of 6-keto-PGF₁α and PGE₂ plasma levels and is not related to an increase of the plasma catecholamine concentration.

Sympathetic stimulation is able to induce prostaglandin formation by activation of α₁-receptors and/or β-receptors according to different tissues.8 In vascular tissues, prostaglandin formation induced by sympathetic stimulation seems to be mediated by α₁-receptors.31,32 The cold pressor test has

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**Table 1. Effects of Subsequent Cold Stimulations on Peripheral Vascular Resistance and Norepinephrine Plasma Concentration**

<table>
<thead>
<tr>
<th>Cold stimulation</th>
<th>PVR (AUC, units/min)</th>
<th>Norepinephrine (AUC, pg/ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>57.8±20.8</td>
<td>1,247±332</td>
</tr>
<tr>
<td>II</td>
<td>78.5±32.3</td>
<td>1,092±294</td>
</tr>
<tr>
<td>III</td>
<td>91.1±34.9</td>
<td>1,216±366</td>
</tr>
<tr>
<td>IV</td>
<td>107.1±33.7*</td>
<td>1,307±210</td>
</tr>
<tr>
<td>V</td>
<td>135.9±49.9†</td>
<td>1,550±630*</td>
</tr>
</tbody>
</table>

Values are mean±SD of 15 subjects. PVR, peripheral vascular resistance; AUC, area under the curve.

* p<0.05 vs. first cold stimulation (Tukey's test).
† p<0.01 vs. first cold stimulation (Tukey's test).
been thought to induce a reflex increase in heart rate and blood pressure consequent to increase of PVR, probably because of the activation of adrenergic fibers and the release of norepinephrine at the neuroeffector junction.33-35

In experimental preparations, locally formed endogenous prostaglandins have been reported as having an inhibitory modulation on sympathetic activation.36-39 Yoshimura et al40 reported that in normotensive humans the pressor response to exogenous norepinephrine was enhanced by indomethacin and suppressed by the infusion of PGE1 at a dose that, by itself, does not significantly decrease blood pressure. Present results appear to be in line with these findings and not only confirm our previous observations in humans13,14 but, above all, provide evidence that an increased vasoconstrictor response may result from a failure of prostaglandin modulation. Indeed, even if after repeated stimulations the decrease and exhaustion of prostaglandin formation is associated with an increase of plasma norepinephrine expressed as area under the curve, this increase did not correlate with the increase in limb vascular resistance consequent to the repeated cold application. On the contrary, the increased vascular responses were significantly related to the progressive decrease and exhaustion of both prostaglandins in venous blood. Prostaglandin blockade induced by declofenac administration caused, from the first cold application, a pattern of the vasoconstrictor response

<table>
<thead>
<tr>
<th>Cold stimulation</th>
<th>AUC (pg/ml/min)</th>
<th>MDC (pg/ml)</th>
<th>AUC (pg/ml/min)</th>
<th>MDC (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>43.4±14.7</td>
<td>19.6±5.2</td>
<td>34.8±14.4</td>
<td>18.4±5.1</td>
</tr>
<tr>
<td>II</td>
<td>44.6±22.0</td>
<td>16.9±5.3</td>
<td>27.9±18.1</td>
<td>17.2±6.8</td>
</tr>
<tr>
<td>III</td>
<td>18.1±3.6*</td>
<td>13.5±1.7*</td>
<td>32.8±13.9</td>
<td>18.3±5.5</td>
</tr>
<tr>
<td>IV</td>
<td>2.0±2.6*</td>
<td>5.9±1.0*</td>
<td>13.8±9.6*</td>
<td>11.6±4.8*</td>
</tr>
<tr>
<td>V</td>
<td>1.0±2.3*</td>
<td>5.6±0.9*</td>
<td>0.0±0.0*</td>
<td>5.0±0.0*</td>
</tr>
</tbody>
</table>

Values are mean±SD of 15 subjects. 6-Keto-PGF1α, 6-ketoprostaglandin F1α; PGE2, prostaglandin E2; AUC, area under the curve; MDC, maximum detected concentration.

*p<0.001 vs. first cold stimulation (Tukey’s test).
and plasma prostaglandin and norepinephrine changes similar to that observed at the fourth or fifth cold application in untreated subjects, when prostaglandins were no longer detectable in plasma. Thus, the exhaustion of prostaglandin formation, as it occurred after repeated sympathetic stimulation, or the blockade of prostaglandin formation, as that after diclofenac administration, resulted in an increased vasoconstrictor response much greater than could have been expected from changes in the norepinephrine concentration in plasma.

Experimental observations gave evidence that the prostaglandins involved in modulating sympathetic stimulation were mainly PGE₂ and PGI₂.²¹,²² PGE₂ seemed to act at a prejunctional level by reducing

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**TABLE 3. Effects of Subsequent Cold Stimulations on Norepinephrine Plasma Concentration Expressed As Area Under the Curve in Time During Placebo and Diclofenac Treatment (200 mg/day p.o. for 3 days)**

<table>
<thead>
<tr>
<th>Cold stimulation</th>
<th>Placebo (AUC, pg/ml/min)</th>
<th>Diclofenac (AUC, pg/ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>902±242</td>
<td>1,181±268</td>
</tr>
<tr>
<td>II</td>
<td>935±236</td>
<td>1,288±289</td>
</tr>
<tr>
<td>III</td>
<td>917±257</td>
<td>1,623±480*†</td>
</tr>
<tr>
<td>IV</td>
<td>1,220±276</td>
<td>1,612±510*</td>
</tr>
<tr>
<td>V</td>
<td>1,619±584‡</td>
<td>1,628±616*</td>
</tr>
</tbody>
</table>

*Values are mean±SD of eight subjects.

†p<0.05 vs. first cold stimulation with diclofenac treatment (Tukey's test).

‡p<0.05 vs. third cold stimulation with placebo treatment (Tukey's test).

Values are mean±SD of eight subjects.

*p<0.05 vs. first cold stimulation with diclofenac treatment (Tukey's test).

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**FIGURE 4. Correlations between the changes in peripheral vascular resistance (PVR) and in prostaglandin plasma concentration after five repeated sympathetic stimulations (I-V). Both parameters are expressed as area under the curve (AUC) with respect to time. Each point (cold application) is the mean value of 15 subjects. For the clarity of the figure, SEM has been reported instead of SD. 6-K-PGF₁α, 6-ketoprostaglandin F₁α; PGE₂, prostaglandin E₂.**

**FIGURE 5. Effects of subsequent and close adrenergic stimulations on plasma concentrations of the catecholamines norepinephrine (○-●) and epinephrine (●-●). The maximum detected concentrations of both catecholamines were unaffected by the replication of the stimulus. Data are mean±SD of 15 subjects. The black bars indicate the duration of cold application.**
norepinephrine outflow evoked by sympathetic stimulation,37-39 whereas PGI₂ had been reported to act mainly at the effector site.43-46 Our experiments did not allow us to identify the exact mechanism of prostaglandin modulation on adrenergic response. The progressive decrease of plasma concentration of PGE₂ but not of PGI₂ was related to the increase of norepinephrine plasma concentration. This seemed to suggest an inhibitory activity of PGE₂, mainly on norepinephrine outflow at a prejunctional level.

The exhaustion of prostaglandin synthesis after repeated and proximate cold applications might have been due to the exhaustion of the available pool of arachidonic acid as in vitro experiments seem to suggest. In bovine or human cultured endothelial and smooth muscle cells, repeated stimulations with ionophore A23183 produced an exhaustion of PGI₂ synthesis that was restored after the addition of arachidonic acid.16

In conclusion, it can be said that repeated and proximate sympathetic stimulations, inducing an exhaustion of prostaglandin modulating mechanism, can result in an exaggerated and inappropriate vasoconstrictor response when compared with the amount of released norepinephrine. Thus, when the braking prostaglandin effect is failing, an excessive vasoconstrictor response may take place even from mild sympathectomized stimulations. This may be clinically relevant in patients with angina pectoris who have an impaired prostaglandin formation47 or in subjects who undergo repeated sympathetic environmental stimulation.

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References


**KEY WORDS** • prostaglandins • prostaglandin modulation • sympathetic stimulation • catecholamines
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