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# STIMULATION OF PROSTACYCLIN PRODUCTION IN BLOOD VESSELS BY THE ANTITHROMBOTIC DRUG SULOCTIDIL

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**Abstract**—Suloctidil is a calcium antagonist with vascular relaxing activity and an antithrombotic agent: its antiplatelet action has been demonstrated *in vivo*, but is difficult to reproduce *in vitro* and the mechanism of this effect remains unknown. We have observed that suloctidil (10 $\mu$M) stimulated the release of prostacyclin ($PGI_2$) from the rabbit aorta, the dog vena cava and the dog portal vein, *in vitro*. This effect could be explained by an increased mobilization of free arachidonic acid. Neither the inactive congener CP894S, nor the two calcium channel antagonists, verapamil and flunarizine, reproduced the stimulatory effect of suloctidil. Suloctidil acted selectively on the vascular endothelium: it stimulated the release of $PGI_2$ from bovine aortic and human umbilical vein endothelial cells, but neither from the de-endothelialized rabbit aorta nor from the bovine aortic media. The stimulatory effect of suloctidil on the release of the platelet inhibitor $PGI_2$ from the vascular endothelium might contribute to the known antiplatelet and antithrombotic activity of this drug.

Suloctidil [1-(4-isopropylthiophenyl)-2-*n*-octylaminopropanol] is a vascular antispasmodic and an antithrombotic agent [1]. The relaxing effect of suloctidil on vascular smooth muscle can be explained by its activity as calcium antagonist [2]. The antithrombotic action of suloctidil has been demonstrated in a variety of animal models of thrombosis: it decreases the incidence of occlusive thrombi in the femoral artery of dogs, following a localized removal of the endothelium, and reduces the incidence and severity of experimental hepatic thrombophlebitis in rats [3]. This antithrombotic activity can be explained, at least partially, by an inhibitory effect on platelet aggregation, which has been demonstrated *in vivo* in animals and man: oral suloctidil reduces the spontaneous formation of circulating platelet aggregates in aged breeder rats [4], normalizes the reduced platelet survival time in patients bearing prosthetic heart valves [5, 6] and reduces the plasma level of $\beta$-thromboglobulin in patients with intermittent claudication [7]. It has been very difficult to reproduce *in vitro* the antiplatelet effect of suloctidil observed *in vivo*: whereas the *ex vivo* aggregation, induced by threshold concentrations of collagen in platelet-rich plasma samples from volunteers, was inhibited after suloctidil ingestion, very large concentrations (>10 $\mu$M) of suloctidil produced only a slight inhibition of platelet aggregation *in vitro* [8]. The only direct effect of suloctidil on platelets, which has been detected so far, is a depletion of serotonin and the blockade of serotonin uptake [4, 8, 9]: the mechanism of the antiplatelet activity of suloctidil remains thus unknown. In this paper, we show that suloctidil induces a large and prolonged stimulation of the release of prostacyclin ($PGI_2$)—a potent inhibitor of platelet aggregation [10]—from the aortic endothelium and other vessels.

## MATERIALS AND METHODS

*Preparation and incubation of vessel rings.* Rabbits weighing ~3 kg were killed by a blow on the neck and the aorta was quickly dissected from the iliac bifurcation up to the arch, trimmed free of fat and connective tissue, and cut into rings (±3 mm large). The rings were incubated at 37°, under constant shaking (80 rpm), in a medium of the following composition: 124 mM NaCl; 5 mM KCl; 1.25 mM $MgSO_4$; 1.45 mM $CaCl_2$; 1.25 mM $KH_2PO_4$; 25 mM Hepes buffer, pH 7.4; 8 mM glucose. For the measurement of arachidonic acid release, the same medium was used, but it contained in addition indomethacin (1 $\mu$g/ml) and bovine serum albumin (BSA: 1 mg/ml). The ratio of tissue weight to medium volume was roughly 25 mg/2 ml. In some experiments, the rings were opened by a longitudinal incision and the intimal face of the resulting strips was scraped with a scalpel. The rings of rabbit aorta were incubated for several 30-min or 60-min periods, and the medium was collected and replaced at the end of each period. Within an experiment (performed with one aorta), each experimental condition was tested in triplicate. Strips of dog inferior vena cava and dog portal vein were incubated in the same conditions.

*Preparation and culture of endothelial cells.* Bovine aortic endothelial cells were obtained by mild collagenase digestion of aorta excised from freshly slaughtered cows, as previously described [11, 12]. These cells were cultured on 94 mm Petri dishes in a medium of the following composition: Dulbecco's modification Eagle's medium (DMEM: 60%), Ham $F_{12}$ medium (20%), fetal calf serum (20%), glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 $\mu$g/ml), amphotericin B (2.5 $\mu$g/ml). At con-

fluency, they were detached by a 5-min incubation in a Ca- and Mg-free Hanks buffer containing trypsin (10 mg/dl) and EDTA (1 mM) and subcultured in 35 mm Petri dishes. Endothelial cells from human umbilical vein were obtained and cultured as described [13]. With both types of cells, the experiments were performed using confluent monolayers ($\pm 10^6$ cells/dish) between passage 2 and 5. The culture medium was removed and, after rinsing, the cells were incubated for 3 periods of 60 min in 1 ml DMEM: this medium was collected and changed at the end of each period. Within an experiment, each condition was tested in triplicate.

*Preparation and culture of bovine aortic media explants.* After removal of the intima and the adventitia from an aorta, excised from a freshly slaughtered cow, the media was cut into small fragments (1–2 $mm^2$). Four to five such explants (20–50 mg) were put in 60 mm Petri dishes and cultured in 2.5 ml of the same medium which was used for culturing endothelial cells (see above). After 24 hr, the medium was removed, the explants were rinsed and incubated for three 60-min periods in 2.5 ml DMEM: the medium was collected and changed at the end of each period.

*Prostaglandin radioimmunoassay (RIA).* The production of $PGI_2$* was measured by the RIA of its stable degradation product, prostaglandin 6-keto-$F_{1\alpha}$ (6-K-$PGF_{1\alpha}$), performed directly in the incubation medium, without extraction and chromatography. A rabbit antiserum was raised against 6-K-$PGF_{1\alpha}$ coupled to BSA, as described [14]: the limit of detection was 16 pg and the cross-reactions were 1.2% with $PGF_{2\alpha}$, 0.3% with $PGE_2$ and <0.1% with thromboxane $B_2$. 100 $\mu$l aliquots of incubation media, $^3$H-6-K-$PGF_{1\alpha}$ (11,000 dpm), anti 6-K-$PGF_{1\alpha}$ antiserum (final dilution: $10^{-4}$) and bovine gamma globulins (0.25 g/dl) in Tris buffer (50 mM, pH 7.4) were incubated in a total volume of 0.4 ml for 60 min at room temperature. Then 0.4 ml of a cold 25% (w/w) solution of polyethylene glycol was added to separate bound and free antigen. In order to confirm that the product measured was authentic 6-K-$PGF_{1\alpha}$, RIA was repeated after purification of a few samples by reversed-phase-high-performance liquid chromatography, as described [15].

*Assay of free arachidonic acid release.* Free arachidonic acid was measured by gas liquid chromatography (GLC) with electron capture detection (ECD). Incubations of aortic rings were performed in a medium containing BSA (1 mg/ml) and indomethacin (1 $\mu$g/ml). After the addition of 1 $\mu$g docosahexaenoic acid as internal standard, the incubation medium was extracted with 1 vol. ethyl acetate. The free fatty acids were converted into pentafluorobenzyl esters by a modification of the method of Wickramasinghe *et al.* [16]. To the dry residue of the extract were added 290 $\mu$g pentafluorobenzylbromide (in 5 $\mu$l acetonitrile) and 44 $\mu$g diisopropylethylamine (in 5 $\mu$l acetonitrile). After 5 min at 40° and evaporation of the solvent under nitrogen, the samples were redissolved in 50 $\mu$l hexane [17]. Direct GLC analysis was performed in a Varian instrument (model 3700, Varian Associates, Palo Alto, CA) equipped with a $^{63}$Ni ECD. Two-metre columns of 3% OV-1 on Gas Chrom Q (Applied Science Laboratories, Waltham, MA) were used isothermally at 235°.

*Statistical analysis.* The statistical significance of observed differences was established using the two-way analysis of variance with a general mixed model for unbalanced data: computations were performed with the use of the $P_3V$ program of the BMDP statistical software [18].

*Materials.* Suloctidil and CP894S were provided by Continental Pharma; flunarizine was a gift from Janssen Pharmaceutica and indomethacin was given by Merck Sharp & Dohme. $^3$H-6-K-$PGF_{1\alpha}$ was purchased from Amersham. Arachidonic acid, docosahexaenoic acid and diisopropylethylamine were obtained from Sigma Chem. Co. BSA and pentafluorobenzyl bromide were purchased respectively from Calbiochem—Behring Corp and Pierce Chemical Co. DMEM, Ham $F_{12}$, glutamine, penicillin, streptomycin, amphotericin B and collagenase type II were purchased from Flow Laboratory. Fetal calf serum was obtained from Gibco.

## RESULTS

At a 10 $\mu$M concentration, suloctidil induced a tremendous stimulation of $PGI_2$ release from rabbit

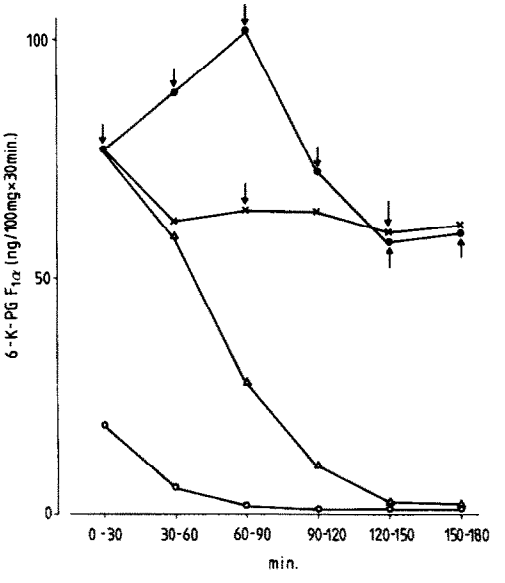


Fig. 1. Time course of the stimulatory effect of suloctidil on the release of $PGI_2$ from the rabbit aorta *in vitro*. Rings of rabbit aorta were incubated for six 30-min periods: the medium was collected and changed at the end of each period. Suloctidil (10 $\mu$M) was added as indicated by the arrows: —○—, control; —●—, suloctidil (10 $\mu$M) present throughout the experiment; —△—, suloctidil (10 $\mu$M) present only during the 1st period; —×—, suloctidil (10 $\mu$M) present during periods 1, 3 and 5. Results represent the amount of 6-K-$PGF_{1\alpha}$ accumulated in the incubation medium (mean of triplicate determinations, in one representative experiment out of five).

* Abbreviations used: $PGI_2$, prostacyclin; 6-K-$PGF_{1\alpha}$, prostaglandin 6-keto-$F_{1\alpha}$; BSA, bovine serum albumin; RIA, radioimmunoassay; DMEM, Dulbecco's modification Eagle's medium; GLC, gas liquid chromatography; ECD, electron capture detection.

aorta rings (Fig. 1). This effect was observed in each of the 26 experiments which were performed. It displayed a characteristic rise with time (Fig. 1). The amplitude of the stimulation was only 2.5 ± 0.5-fold during the 1st 30 min incubation period and increased to 13 ± 3-fold and 19 ± 3-fold during the second and third periods of continuous exposure to the drug, respectively (mean ± SE, seven experiments). Continuous exposure of rabbit aorta rings to suloctidil (10 $\mu$M) elicited a sustained stimulation, which reached its maximum during the second hour of incubation, slowly declined thereafter, but was still detectable after 3 hr (Fig. 1) and even 4 hr (not shown). The stimulation was reversible following removal of the drug; the intermittent readdition of suloctidil was sufficient to maintain the rate of $PGI_2$ release at a stimulated level (Fig. 1). The concentration–response curve was very steep: no stimulatory effect could be detected at or below 2 $\mu$M, whereas at 8 $\mu$M soloctidil produced a maximal stimulation (Fig. 2). At 2 $\mu$M, suloctidil—which was thus devoid of any effect *per se*—potentiated and prolonged the stimulation of $PGI_2$ release induced by submaximal concentrations of ADP [15] or acetylcholine [19] (Fig. 3). The interactions of suloctidil with either ADP or acetylcholine, during both periods of incubation, were highly significant:

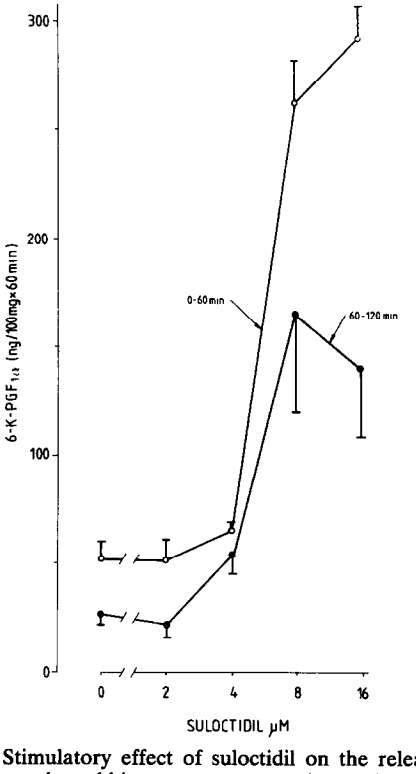


Fig. 2. Stimulatory effect of suloctidil on the release of $PGI_2$ from the rabbit aorta: concentration–action curve. Rings of rabbit aorta were incubated for two periods of 60 min in the presence of various concentrations of the drug. Results represent the amount of 6-K-$PGF_{1\alpha}$ accumulated in the incubation medium at the end of the first (—○—) and of the 2nd period (—●—) (mean ± SD of triplicate determinations in one representative experiment out of three).

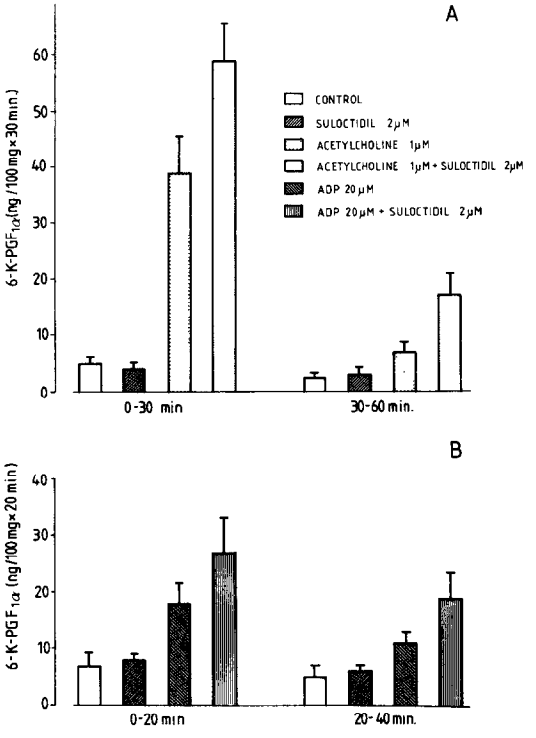


Fig. 3. Potentiation by suloctidil of the cholinergic (A) and purinergic (B) stimulations of $PGI_2$ release from the rabbit aorta. Rings of rabbit aorta were incubated for two periods of 20 or 30 min with the various agents. Results represent the amount of 6-K-$PGF_{1\alpha}$ accumulated in the incubation medium at the end of each period (mean ± SD : six experiments for A and four experiments for B, triplicate determinations in each experiment).

$P < 0.001$ in each case (Fig. 3). The release of $PGI_2$ induced by suloctidil was blocked by indomethacin (not shown). Suloctidil increased the mobilization of free arachidonic acid in rabbit aorta rings, with the same time course as the stimulation of $PGI_2$ release (Fig. 4). The specificity of suloctidil action was assessed in two ways. The chemical analog CP894S, which is devoid of the characteristic pharmacological properties of suloctidil but shares its physico-chemical effect on membrane fluidity [1, 20], was completely inactive, even at 50 $\mu$M (data not shown). Neither verapamil, nor flunarizine—which are both calcium antagonists like suloctidil—did stimulate $PGI_2$ release from rabbit aorta rings (Tables 1 and 2). The stimulatory effect of suloctidil on $PGI_2$ release was not restricted to the rabbit aorta: it was also observed in the dog inferior vena cava (not shown) and the dog portal vein (Fig. 5).

Both the endothelial cells and the smooth muscle cells of the vessel wall generate $PGI_2$ [10, 21]: the following experiments were performed to determine in which of these cells suloctidil stimulated $PGI_2$ production. Mechanical removal of the endothelium *in vitro* has been shown to induce an immediate and transient stimulation of $PGI_2$ release from the rabbit aorta: it is believed that the rapid decline of this stimulation is due to the self-inactivation of cyclooxygenase [17]. The increases of $PGI_2$ release from

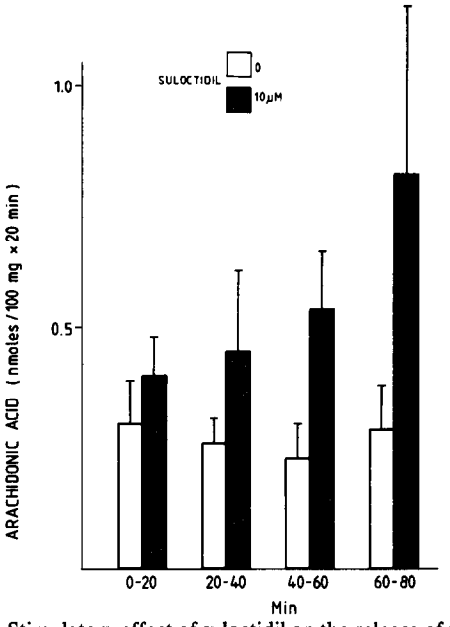


Fig. 4. Stimulatory effect of suloctidil on the release of free arachidonic acid in the rabbit aorta. Rings of rabbit aorta were incubated for four 20-min periods in a medium containing BSA (1 mg/ml) and indomethacin (1 μg/ml). The medium was collected and replaced at the end of each period. Arachidonic acid was measured by GLC-ECD, as described in Methods. Results represent the mean ± SD of six measurements (duplicate determinations in three separate experiments).

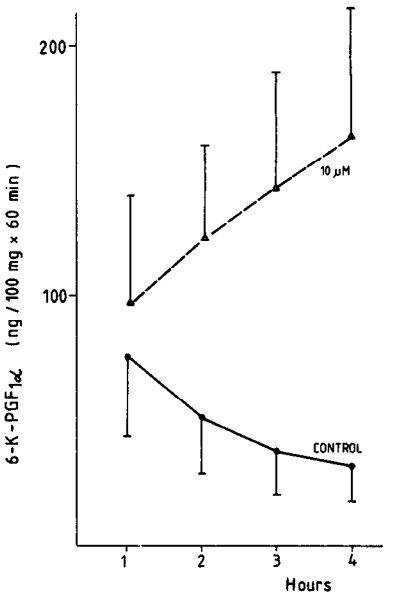


Fig. 5. Kinetics of $PGI_2$ release from the dog portal vein stimulated by suloctidil (10 μM). Strips of dog portal vein were incubated for four 1-hr periods, with (—△—) or without (—●—) suloctidil (10 μM): the medium was collected and replaced at the end of each period. Results represent the amount of 6-K-$PGF_{1\alpha}$ accumulated in the medium (mean ± SD of six measurements, duplicate determinations in three separate experiments).

Table 1. Comparison between the effects of verapamil and suloctidil on $PGI_2$ release from the rabbit aorta

| | 0–60 min | 60–120 min |
|---|---|---|
| — | 15 ± 6 | 9 ± 4 |
| Verapamil 5 μM | 15 ± 6 | 6 ± 2 |
| Verapamil 50 μM | 8 ± 3 | 4 ± 3 |
| Suloctidil 10 μM | 162 ± 49 | 261 ± 96 |

Rings of rabbit aorta were incubated for two periods of 60 min in the presence of these drugs: the medium was changed between the two periods. The results represent the amount of 6-K-$PGF_{1\alpha}$ (ng/100 mg tissue) accumulated in the incubation medium (mean ± SD of six measurements, triplicate determinations in two separate experiments).

Table 2. Comparison between the effects of flunarizine and suloctidil on $PGI_2$ release from the rabbit aorta

| | 0–60 min | 60–120 min | 120–180 min |
|---|---|---|---|
| | 18 ± 4 | 9 ± 4 | 7 ± 1 |
| Flunarizine 2 μM | 16 ± 11 | 6 ± 5 | 5 ± 3 |
| Flunarizine 10 μM | 25 ± 13 | 16 ± 9 | 18 ± 8 |
| Flunarizine 100 μM | 23 ± 10 | 22 ± 10 | 17 ± 5 |
| Suloctidil 10 μM | 111 ± 16 | 155 ± 14 | 83 ± 18 |

Rings of rabbit aorta were incubated for three periods of 60 min in the presence of these drugs: the medium was changed at the end of each period. The results represent the amount of 6-K-$PGF_{1\alpha}$ (ng/100 mg tissue) accumulated in the incubation medium (mean ± SD of six measurements, triplicate determinations in two separate experiments).

the rabbit aorta induced by de-endothelialization and by exposure to suloctidil were not additive (Fig. 6). The continuous presence of suloctidil did not prevent the progressive fall of $PGI_2$ production in the de-endothelialized aorta: in particular, during the second hour following endothelium removal, the de-endothelialized aorta strips released as much $PGI_2$ in the presence or absence of suloctidil and less than the intact strips exposed to suloctidil (Fig. 6). These data, suggesting that suloctidil stimulates endothelial $PGI_2$, were confirmed by experiments with cultured endothelial cells. Suloctidil stimulated the release of $PGI_2$ from endothelial cells, cultured either from the bovine aorta (Fig. 7) or from the human umbilical vein (not shown). This stimulation had several features in common with that observed in the rabbit aorta: it was very large at 10 μM, but undetectable at 2 μM, its duration was at least 3 hr and the chemical analog CP894S was much less active than suloctidil itself. The endothelial cells started to detach from the dishes after 2 hr of continuous exposure to suloctidil (10 μM). Ionophore A23187 (5 μM) produced endothelial cell detachment after only 30–60 min. In contrast with these results, suloctidil did not stimulate the production of $PGI_2$ in cultured explants of bovine aortic media, a preparation which contains exclusively smooth muscle cells and which is responsive to serotonin [22] (not shown).

## DISCUSSION

Previous studies of the influence of suloctidil on vascular $PGI_2$ have provided discrepant results: the

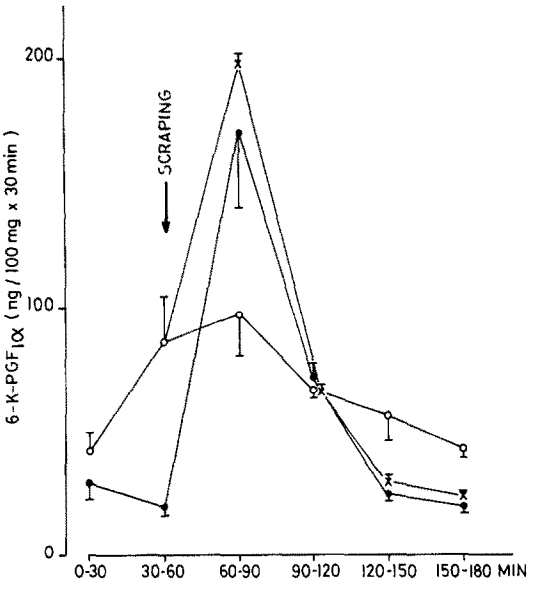


Fig. 6. Lack of suloctidil effect on $PGI_2$ release from the de-endothelialized rabbit aorta. Strips of rabbit aorta were incubated for six 30-min periods with (—○—, —×—) or without (—●—) suloctidil (10 μM): the medium was collected and replaced at the end of each period. At the end of the second period, the intimal surface of some strips (—●—, —×—) was scraped with a scalpel in order to remove the endothelium, whereas others were left intact (—○—). Results represent the amount of 6-K-$PGF_{1\alpha}$ accumulated in the medium at the end of each period (mean ± range of duplicate measurements, in one representative experiment out of four).

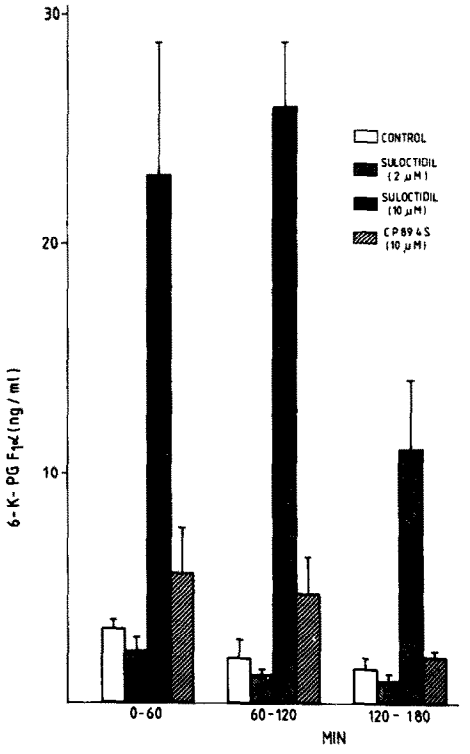


Fig. 7. Stimulatory effect on suloctidil on the release of $PGI_2$ from bovine aortic endothelial cells. These cells were incubated for three 60-min periods in the presence of suloctidil or analog CP894S: the medium was collected and changed at the end of each period. Results represent the amount of 6-K-$PGF_{1\alpha}$ accumulated in the incubation medium (mean ± SD of 6 measurements, triplicate determinations in two separate experiments).

*ex vivo* release of $PGI_2$-like activity from the rat aorta, measured by bioassay, was unchanged after oral intake of suloctidil [23], but was increased following intravenous administration [24].

Our *in vitro* studies have demonstrated a stimulatory action of suloctidil on the vascular production of $PGI_2$, which appears to be a general phenomenon: it was observed with different vessels (aorta, vena cava, portal vein, umbilical vein) in different species (human, canine, bovine and rabbit). Suloctidil exerted its effect selectively on the endothelium and was inactive on vascular smooth muscle. The time course of suloctidil action was completely different from that of other stimuli active on the endothelial cells: whereas ADP action was characterized by an immediate onset and a short duration (~10 min) followed by a period of refractoriness [12], the effect of suloctidil was delayed and could be sustained for several hours, provided the drug was continuously present. The sustained response of the endothelium to suloctidil also differs from the transient biosynthesis of $PGI_2$, limited by cyclooxygenase deactivation, which has been observed in the endothelium stimulated by exogenous arachidonic acid [25, 26] or in the aortic smooth muscle following endothelium removal [17]. The concentration-dependency of suloctidil action was particularly steep: at 10 μM, suloctidil induced huge effects, whereas it was completely inactive at 2 μM. This feature has been observed in the various experimental models studied (rabbit aorta, cultured endothelial cells from bovine aorta and human umbilical vein) and seems therefore to be an intrinsic property of the drug. Other effects of suloctidil are also characterized by steep concentration–action curves: for instance, a threshold inhibitory effect on the norepinephrine-induced contraction of the rat aorta is obtained at 1 μM and 10 μM produces a total inhibition [1]. The stimulatory action of suloctidil on vascular $PGI_2$ was doubly specific. On one hand, it was not mimicked by the analog CP894S: this compound—in which the secondary amine function of suloctidil has been replaced by a thioether function—is much less potent than suloctidil as a calcium antagonist and vascular relaxing agent [2], but increases the fluidity of artificial and natural membranes to a larger extent than suloctidil itself [20]. On the other hand, the two calcium antagonists verapamil and flunarizine were also inactive. Flunarizine was a particularly adequate control, because it has been reported to exert protective effects on endothelial cells [27] and since its behavior towards $Ca^{2+}$ channels is similar to that of suloctidil: increased affinity in skeletal muscle, as compared to brain and heart [1]. The stimulatory effect of suloctidil on $PGI_2$ biosynthesis can be explained by an increased mobilization of free arachidonic acid. The mechanism of this last effect remains unknown: a

role of increased membrane fluidity or of a $Ca^{2+}$ channel blockade seems excluded by the experiments with CP894S and flunarizine, respectively. We have recently observed that suloctidil induced a slow and sustained efflux of $^{45}Ca$ from prelabeled bovine aortic endothelial cells (E. Raspe and J. M. Boeynaems, unpublished data). As reflected by the stimulatory effect of ionophore A23187 [28, 29], the cytoplasmic concentration of free $Ca^{2+}$ plays a major role in the control of endothelial $PGI_2$ synthesis. Moreover, the toxicity of both agents might be explained by an excessive increase of cytosolic $Ca^{2+}$ [30].

The mechanism of the antiplatelet action of suloctidil has not yet been elucidated [1]: the difficulty in reproducing *in vitro* this effect, which has been demonstrated *in vivo*, would be entirely consistent with a mechanism involving an increased release of the unstable platelet inhibitor $PGI_2$ from the vascular endothelium [10]. The disparity between the concentrations of suloctidil required to obtain this effect *in vitro* (5–10 $\mu$M) and the plasma levels reached during chronic treatment (5–20 nM: [31]) constitutes an argument against this hypothesis. However, in most tissues suloctidil concentrations are higher than in plasma [1]. Furthermore, the same type of disparity is observed for *in vitro* effects likely to reflect the vascular antispasmodic activity obtained *in vivo*: the $IC_{50}$ are 0.9 $\mu$M for the displacement of [$^3$H]nitrendipine bound to cerebral cortex membranes [2] and 2.0 $\mu$M for the inhibition of rat aorta contractions induced by $Ca^{2+}$ after depolarization [32]. Suloctidil is extensively metabolized in the liver [1], so that its concentration in portal blood following oral intake is likely to be much higher than its level in the systemic circulation. Our observation that suloctidil stimulated $PGI_2$ release from the portal vein suggests therefore the possibility of a presystemic mechanism of action: platelets would be inhibited by $PGI_2$ while they flow through the portal circulation during the absorption of suloctidil from the gut. Although highly speculative, this mechanism would be similar to the presystemic inhibition of platelets by low doses of aspirin, recently described [33]; one major difference is that aspirin action is irreversible, whereas the inhibition by presystemic $PGI_2$ would be of short duration, but could be repeated each time the platelets flow through the portal circulation.

So far, three main drugs have been reported to increase the vascular synthesis of $PGI_2$: nitroglycerin [34], dipyridamole [35, 36] and nafazatrom [37]. The initially reported effect of nitroglycerin could not be reproduced later [38]. The antithrombotic agent nafazatrom is a radical scavenger which can protect cyclooxygenase and prostacyclin synthase from oxidative inactivation [39]: its action is rather to prolong the synthesis of $PGI_2$ stimulated by another agent than to induce it *per se* [40]. The mechanism of dipyridamole action would be similar to that of nafazatrom [39]: we recently observed that dipyridamole does not stimulate the release of $PGI_2$ from endothelial cells (bovine aorta and human umbilical vein), but prolongs the transient release of $PGI_2$ induced by removing the endothelium from the rabbit aorta [41]. Whether suloctidil itself is active *in vivo* or not, our *in vitro* study provides a starting point for the development of $PGI_2$-stimulatory drugs. However, the therapeutic benefit of a drug producing a large, prolonged and generalized release of $PGI_2$ from the vascular endothelium is questionable, because such a drug could have an undissociable toxic effect on the endothelial cells and produce side-effects (f.i. hypotension) similar to those of exogenous $PGI_2$. The most interesting action of suloctidil, observed in this study, might be its ability to potentiate and prolong the ADP-induced release of $PGI_2$, at a concentration too low to produce a stimulation *per se*: this would thus increase $PGI_2$, only where and when it is needed, in response to platelet activation.

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