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SELECTIVE INHIBITION BY MINOXIDIL OF PROSTACYCLIN PRODUCTION BY CELLS IN CULTURE

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Abstract—The effect of minoxidil on arachidonic acid metabolism by cells in culture was studied. In bovine aorta endothelial cells, treatment with minoxidil in the presence of various stimulators of arachidonic acid metabolism was accompanied by a dose-dependent inhibition of prostacyclin production (measured as 6-keto-prostaglandin $F_{1\alpha}$). Synthesis of the other cyclooxygenase products (prostaglandins E_2, $F_{2\alpha}$ and thromboxane) was not inhibited. When the bovine aorta endothelial cells were stimulated by the Ca^{2+} ionophore A-23187, the inhibition was seen as early as 2 min. Minoxidil also inhibited prostacyclin production by a second cell line of bovine aorta endothelial cells (the established CPAE cell line), bovine aorta smooth muscle cells, porcine aorta endothelial cells, and rat liver cells (the C-9 cell line)—the latter, less effectively. Again, formation of all the other cyclooxygenase products studied was not inhibited. Minoxidil did not affect significantly prostaglandin E_2 and $F_{2\alpha}$ production by newborn rat keratinocytes (the NBR cell line)—a cell that does not produce PGI_2. The clinical, biochemical, and pharmacologic implications are discussed.

Minoxidil [6-(1-piperidinyl)-2,4-pyrimidinediamine 3-oxide] is a potent antihypertensive agent in man [1, 2]. The biochemical mechanism by which minoxidil produces its antihypertensive effects is unknown. It is said to be a direct acting smooth muscle vasodilator [3, 4]. Evidence supporting this claim has been of two types, both indirect. In whole blood vessel preparations, the effects of minoxidil are not altered by treatment with adrenergic receptor agonists or calcium channel blockers [3]. Also, in whole vessel preparations where the endothelium has been removed mechanically, treatment with minoxidil is associated with vasodilation [4]. There are no published studies examining direct effects of minoxidil on metabolism of smooth muscle cells in culture which might explain its effects.

Minoxidil has the unique side effect of increased growth of vellus hair [5, 6], and thus has been used in the treatment of male pattern hair loss [7–9] and alopecia areata [10]. In view of these findings, the effects of minoxidil on some possibly related activities have been studied. Minoxidil has been shown to slow the senescence of cultured human keratinocytes [11] at doses of 0.03 to 0.3 mM and to stimulate a time-dependent increase in tritiated thymidine incorporation in cultured mouse keratinocytes [12] at a dose of 0.025 mM. It has also been shown, at 0.5 mM, to suppress the activity of lymphocytes isolated from human peripheral blood as assayed by DNA synthesis in response to phytohemagglutinin stimulation [13]. Finally, in doses ranging from 25 μM to 1 mM, minoxidil has been shown to inhibit lysylhydroxylase in human fibroblasts [14]. No unifying concept of biochemical action has been formulated to explain these wide-ranging effects.

MATERIALS AND METHODS

Materials. All reagents were stored at −20° as concentrated solutions and diluted into MEM§ for experiments. Bradykinin and melittin (Sigma Chemical Co., St. Louis, MO) were stored in MEM at 0.1 mg/ml; minoxidil (a gift from the Upjohn Co.) was stored in MEM at 1 mg/ml; and endotoxin from *Shigella flexneri* (RIBI ImmunoChem Research Inc., Hamilton, MT) was stored at 100 μg/ml MEM. The Ca^{2+} ionophore, A-23187, TPA, and rutin (Sigma) were stored at 1, 0.1 and 100 mg/ml, respectively, in DMSO. Arachidonic acid (Sigma) was made at 5 mg/ml DMSO prior to each experiment. All reagents stored in DMSO were diluted into MEM at least 1:1000, a concentration of DMSO which has no effect on arachidonic acid metabolism in any of the cell lines used in our experiments.

Culture and cell lines. Cell lines were maintained at 37° in an atmosphere of 95% air, 5% CO_2 in 100 mm culture dishes (Falcon, Cockeysville, MD) in Eagle's MEM (Gibco Laboratories, Grand Island, NY) supplemented with 10% (v/v) fetal bovine serum. Bovine aorta endothelial cells were isolated as previously described [15, 16], and their identity was authenticated by the cobblestone appearance of confluent cultures under light microscopy [15] and positive immunofluorescent staining for Factor VIII [17]. This cell line was obtained from Dr. K. C.

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§ Abbreviations: AA, arachidonic acid; DMSO, dimethyl sulfoxide; EGF, epidermal growth factor; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; TxB_2, thromboxane B_2; IC_{50}, concentration required for 50% inhibition; MEM, minimal essential medium; PG, prostaglandin; RIA, radioimmunoassay; and TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

Hayes, Department of Biology, Brandeis University. The C-9 rat liver cell line and the bovine aorta endothelial line (CPAE) were obtained from the American Type Culture Collection, Rockville, MD. Newborn rat cultured keratinocytes were isolated and grown as previously described [18]. Their authenticity as keratinocytes was confirmed by biochemical and immunologic detection of cytokeratines and filagrin, proteins unique to squamous epithelia. The bovine aortic smooth muscle cells were cultured and characterized as reported [19]. The porcine endothelial cells were obtained from Dr. Michael Moskowitz, Department of Neurosurgery, Massachusetts General Hospital, Boston, MA.

Experimental procedure. One day prior to experiments, subconfluent cultures were treated with 0.25% trypsin-EDTA (Gibco Laboratories, Grand Island, NY) and seeded onto 35 mm culture dishes (Falcon, Cockeysville, MD). The plating densities for the endothelial cells, the keratinocytes and the smooth muscle cells varied from 0.5 to 4×10^5/35 mm dish and the density for the C-9 cells was 4×10^5/35 mm dish. These freshly seeded cultures were incubated overnight to allow for cell attachment. The plating efficiencies for all of the cells in this medium were not determined. At these subconfluent cell densities, production of arachidonic acid metabolites is maximal [20], and these products are not further metabolized to 15-keto- or 13,14-dihydro-15-keto-compounds [21]. The cells were then washed twice with MEM and incubated in MEM in the absence and presence of the modulators. Minoxidil was not toxic at the 470 μM level when tested by trypan blue exclusion [22]. At designated times culture fluids were collected, centrifuged at 1500 *g* for 30 min at 4° to remove any cellular debris, and analyzed by RIA. In many of the experiments, the cultures of treated and untreated cells were incubated for 20 or 22 hr before being assayed by RIA. This time was chosen for convenience since the time–course experiments never have shown loss of serologically determined product from the culture fluid either because of further metabolism or reincorporation into cells.

RIA. RIA was performed as previously described with antibodies of known specificities [23]. The limits of sensitivity for the PGE_2, $PGF_{2\alpha}$, 6-keto-$PGE_{1\alpha}$ and TxB_2 immune systems are 0.009, 0.012, 0.018 and 0.005 ng/ml respectively. The antibody to 6-keto-$PGF_{1\alpha}$ reacted <1% with 6-keto-PGE_1 (unpublished data). Minoxidil, when tested at a concentration of 0.5 mM, did not react with any of the antibodies.

HPLC. For HPLC separation of arachidonic acid metabolites, groups of fifteen dishes were incubated as described above for 20 hr. The culture fluids were pooled, assayed by RIA, and then extracted and the extracts subjected to HPLC analyses. The extraction was as follows and gave a yield of approximately 70%: 3 vol. of ethanol was added to the pooled culture fluids and, after 1 hr at room temperature, this 75% ethanol suspension was clarified by centrifugation. The supernatant fluids were reduced to solid by vacuum distillation. The residue was dissolved in 200 μl ethanol, clarified by centrifugation, and injected for HPLC. Reverse phase HPLC was performed on a μBondapak phenyl column (Waters Assoc.; 7.8 to 300 mM) using a starting solvent of 0.6% amyl alcohol, 6% methanol, and 93.4% 10 mM K_2HPO_4, pH 7.4. Elution was performed using a linear gradient of increasing methanol concentration to a final solution of 0.6% amyl alcohol, 99.4% methanol [24]. Fractions (1 ml) were collected, and 20–50 μl of each fraction was analyzed by RIA [25].

Presentation of data. The data in the tables and figures are from one experiment. Such results were reproduced in all experiments, and all experiments were performed more than twice. The absolute levels of arachidonic acid metabolites produced by cells in culture did vary from experiment to experiment, but what remained reproducible in each experiment were the relative differences in the levels of cyclooxygenase metabolites produced by the cells stimulated in the presence and absence of minoxidil. The results are presented as means ± SD. Comparisons between prostaglandin levels in the presence and absence of minoxidil were made using Student's *t* test for unpaired observations.

RESULTS

The bovine aorta endothelial cells obtained from Dr. Hayes produce primarily PGI_2 (80–90%) and lesser amounts of PGE_2, $PGF_{2\alpha}$ and thromboxane. The absolute quantities of the assayed products varied from day to day, but the levels of 6-keto-$PGF_{1\alpha}$ were always eight to nine times greater than the sum of all other products assayed. The reasons for this variation are not known, but are probably related to the number of cells in culture. Cultures of these bovine aorta endothelial cells incubated with A-23187 (1 μM), bradykinin (0.1 μM), melittin (1.8 μM), and *S. flexneri* endotoxin (10 μg/ml) were stimulated to produce increased levels of cyclooxygenase products. In the presence of minoxidil, inhibition of 6-keto-$PGF_{1\alpha}$ synthesis was observed (Table 1). Minoxidil did not inhibit PGE_2, $PGF_{2\alpha}$ or TxB_2 production by these cells; it actually increased their syntheses. However, with all cells and with all stimulants and in all of our experiments, the sum of the increases *never* equalled the mass of the inhibited 6-keto-$PGF_{1\alpha}$ (depending on the stimulant, the sum of these increases varied from 4 to 35% of the inhibited 6-keto-$PGF_{1\alpha}$). The dose-dependent inhibitions of 6-keto-$PGF_{1\alpha}$ by minoxidil after stimulation of arachidonic acid metabolism by A-23187 (1 μM), bradykinin (0.1 μM) and *Shigella* endotoxin (10 μg/ml) are shown in Fig. 1; 50% inhibition was observed at concentrations of 20, 10, and 41 μM respectively. Minoxidil inhibited 6-keto-$PGF_{1\alpha}$ production regardless of the stimulant. In subsequent experiments, in order to inhibit 6-keto-$PGF_{1\alpha}$ production maximally, minoxidil at 235 or 470 μM was used. The effect of minoxidil was rapid. Inhibition of 6-keto-$PGF_{1\alpha}$ was evident after a 2- to 3-min incubation of the bovine aorta endothelial cells with A-23187 and minoxidil (Fig. 2).

The inhibition of 6-keto-$PGF_{1\alpha}$ production by minoxidil after stimulation with melittin was also demonstrated after extraction of the culture fluids followed by their separation by HPLC and RIA of the HPLC fractions. The 6-keto-$PGF_{1\alpha}$ and TxB_2

Table 1. Effect of minoxidil on arachidonic acid metabolism by bovine aorta endothelial cells after stimulation with several compounds*

Reagent	Dose	6-keto-$PGF_{1\alpha}$	TxB_2	PGE_2	$PGF_{2\alpha}$
		(ng/ml)			
MEM	—	0.13	≤0.05	0.05	0.01
+ minoxidil	50 μM	0.07	ND	ND	ND
Bradykinin	0.1 μM	2.35	ND	0.20	ND
+ minoxidil	50 μM	0.36	ND	0.28	ND
A-23187	1 μM	137.0	2.2	20.1	11.4
+ minoxidil	470 μM	3.4	3.5	31.2	15.0
Melittin	1.8 μM	70.0	1.76	14.5	22.5
+ minoxidil	470 μM	1.89	4.0	31.7	29.6
Endotoxin (*Shigella*)	10 μg/ml	1.7	0.02	0.11	0.10
+ minoxidil	470 μM	0.14	0.07	0.36	0.30

* Cells (1×10^5) were incubated for 22 hr, and culture fluids were decanted and analyzed by RIA. The data represent the means of duplicate analyses from a single experiment. Such data have been reproduced in many experiments. ND = not determined.

cochromatographed with authentic 6-keto-$PGF_{1\alpha}$ and TxB_2 (Fig. 3); in the presence of minoxidil the decrease in the mass of 6-keto-$PGF_{1\alpha}$ (4.8 ng) was greater than the increase in mass (1.7 ng) of TxB_2.

The cyclooxygenase activity of these endothelial cells responded as expected to the non-steroidal anti-inflammatory drug indomethacin. Inhibition of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2 and TxB_2 production by indomethacin (56 μM) after stimulation by 1 μM A-23187 was rapid (Fig. 4), and the inhibition of *all* of the cyclooxygenase products by indomethacin was dose-dependent; the IC_{50} was 1.5 μM (Fig. 5). Addition of rutin (150 μM), at a concentration that has been shown to inhibit 9-hydroxydehydrogenase [26], the enzyme that converts PGI_2 to 6-keto-PGE_1 in many cells [27] including endothelial cells [28], to cell cultures incubated with minoxidil (470 μM) and A-23187 (1 μM) for 22 hr had no effect on the inhibition of 6-keto-$PGF_{1\alpha}$ production (Table 2).

Minoxidil inhibited PGI_2 production by bovine aorta smooth muscle cells, porcine aorta endothelial cells and by another cell line of bovine aorta endothelial cells (the established CPAE cell line) after stimulation with 1.0 μM A-23187 (Table 3) and 8.4 nM TPA (Table 4); again, the levels of the minor cyclooxygenase products of these cells (PGE_2, $PGF_{2\alpha}$ and/or TxB_2) were not inhibited, but were increased slightly. Minoxidil also inhibited the PGI_2 production by rat liver cells that had been stimulated by several compounds (Table 5); but the inhibition by minoxidil of A-23187-stimulated PGI_2 production by the rat liver cells was not as effective as that found with the bovine endothelial cells [IC_{50} bovine endothelial cells = 20 μM; IC_{50} rat liver cells = 700 μM as determined by analyses of the inhibition of 6-keto-$PGF_{1\alpha}$

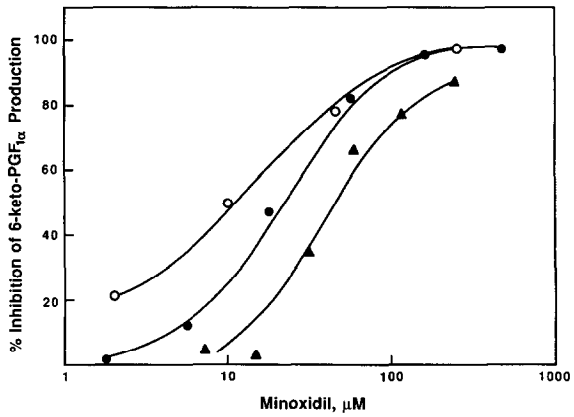

Fig. 1. Inhibition by minoxidil of 6-keto-$PGF_{1\alpha}$ production by 1×10^5 bovine aorta endothelial cells incubated for 20 hr with 1 μM A-23187 (●), 10 μg/ml *Shigella* toxin (▲), and 0.1 μM bradykinin (○).

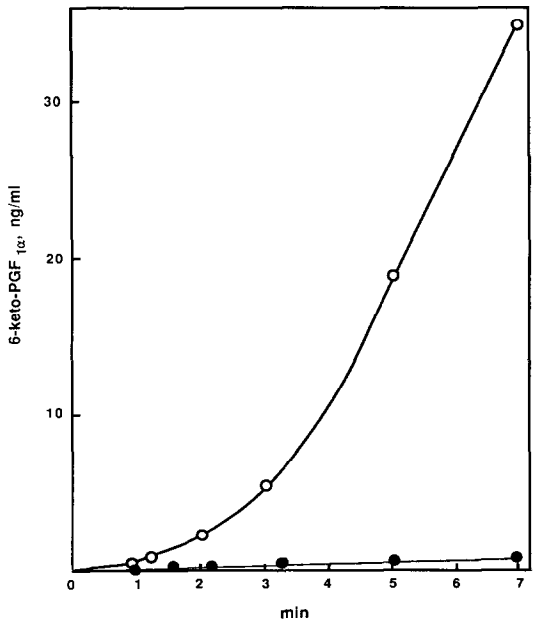

Fig. 2. Production of 6-keto-$PGF_{1\alpha}$ by 1×10^5 bovine aorta endothelial cells as a function of time. Key: (○) 1 μM A-23187; (●) 1 μM A-23187 + 470 μM minoxidil.

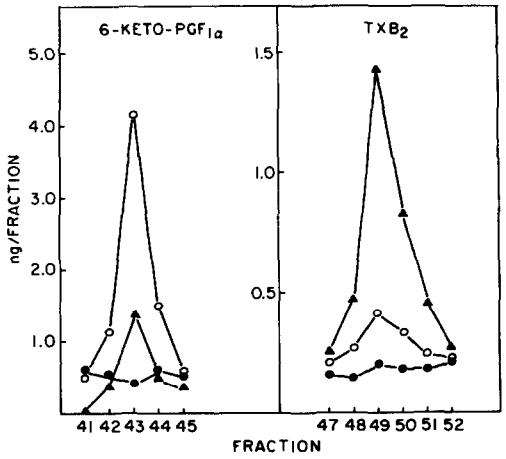

Fig. 3. RIA of HPLC fractions which cochromatographed with authentic 6-keto-$PGF_{1\alpha}$ (fractions 41–45) or TxB_2 (fractions 47–52). Cultures of bovine aorta endothelial cells were incubated for 20 hr with MEM (●), melittin 1.8 μM (○) or melittin 1.8 μM + minoxidil 470 μM (▲).

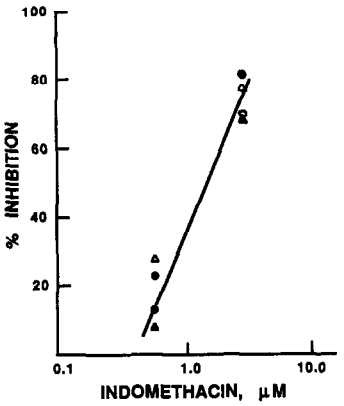

Fig. 5. Inhibition of cyclooxygenase metabolite production by bovine aorta endothelial cells incubated for 22 hr as a function of indomethacin concentration after stimulation by 1 μM A-23187. Key: PGE_2 (△), $PGF_{2\alpha}$ (▲), 6-keto-$PGF_{1\alpha}$ (○), TxB_2 (●).

production stimulated by 1 μM A-23187 by various doses of minoxidil (Fig. 6)].

Because of minoxidil's side effect of increased growth of vellus hair [5, 6], its effect on arachidonic acid metabolism by the keratinocytes was studied. The rat keratinocytes (the NBR cell line) produce PGE_2 and $PGF_{2\alpha}$, not PGI_2 [29]. PGE_2 and $PGF_{2\alpha}$

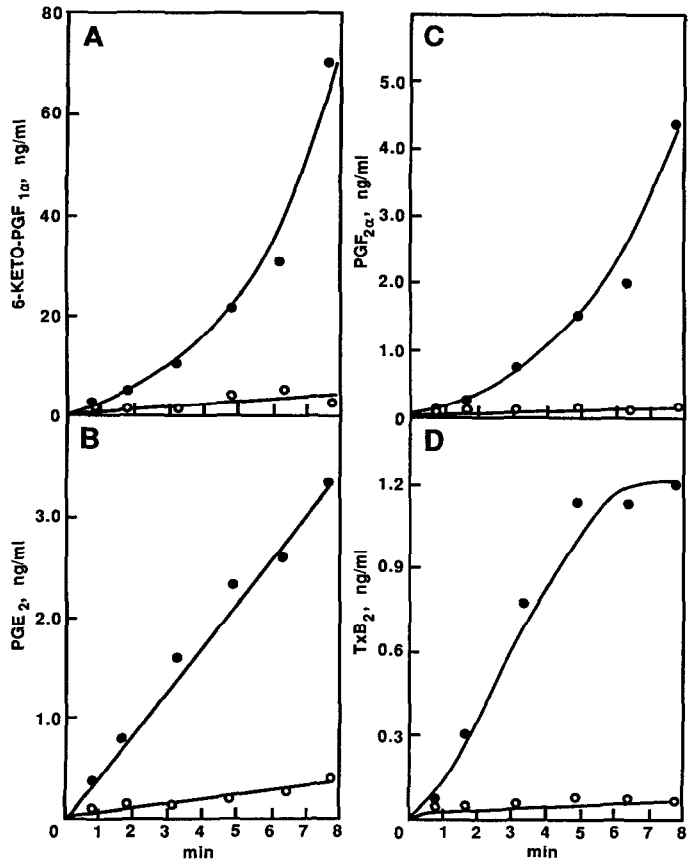

Fig. 4. Cyclooxygenase products produced by bovine aorta endothelial cells after incubation for 20 hr with 1 μM A-23187 (●) or 1 μM A-23187 + 56 μM indomethacin (○). (A) 6-keto-$PGF_{1\alpha}$, (B) PGE_2, (C) $PFG_{2\alpha}$, and (D) TxB_2.

Table 2. Effect of rutin on the inhibition by minoxidil of 6-keto-$PGF_{1\alpha}$ production by bovine aorta endothelial cells*

Reagent	6-ketoPGF$_{1\alpha}$	$PGF_{2\alpha}$ (ng/ml)	TxB_2
MEM	1.2	<0.1	<0.05
Rutin (150 μM)	1.12 ± 0.359 (3)	<0.1 (3)	<0.05 (3)
A-23187 (1 μM)	97.33 ± 15.085 (3)	1.21 ± 0.182 (3)	1.43 ± 0.125 (3)
A-23187 (1 μM) + minoxidil (470 μM)	2.51 ± 0.415 (3)	2.17 ± 0.393 (3)	1.93 ± 0.249 (3)
A-23187 (1 μM) + minoxidil (470 μM) + rutin (150 μM)	1.86 ± 0.272 (3)	2.73 ± 0.047 (3)	1.57 ± 0.236 (3)

* Cells (1×10^5) were incubated in the presence of the indicated reagent. After 22 hr, the culture fluids were collected and analyzed by RIA. Data from duplicate analyses are the means ± SD for the number of dishes in parentheses.

Table 3. Effect of minoxidil on A-23187 stimulated arachidonic acid metabolism by bovine aorta smooth muscle cells, bovine aorta endothelial cells (the CPAE cell line), and porcine aorta endothelial cells*

Cell	Reagent	6-keto-$PGF_{1\alpha}$	PGE_2 (ng/ml)	$PGF_{2\alpha}$
Bovine smooth muscle	MEM	0.33 ± 0.097 (3)†		<0.009‡
	Minoxidil (470 μM)	0.19 ± 0.041 (3)		<0.009
	A-23187 (1 μM)	5.98 ± 1.513 (3)		0.015
	Minoxidil (470 μM) + A-23187 (1 μM)	0.48 ± 0.103 (3)		0.096
	Minoxidil (157 μM) + A-23187 (1 μM)	1.08 ± 0.158 (5)		0.078
	Minoxidil (52 μM) + A-23187 (1 μM)	1.94 ± 0.309 (5)		0.065
Bovine aorta endothelial	MEM	1.35	<0.06	0.03
	Minoxidil (470 μM)	0.10	0.17	0.04
	A-23187 (1 μM)	36.0 ± 2.83 (3)	0.17	0.06
	Minoxidil (470 μM) + A-23187 (1 μM)	0.98 ± 0.041 (3)	1.09	0.21
Porcine aorta endothelial	MEM	0.30 ± 0.089 (4)	<0.1	<0.009
	Minoxidil (470 μM)	0.14 ± 0.036 (4)	<0.1	<0.009
	A-23187 (1 μM)	49.0 ± 5.05 (4)	2.04 ± 0.167 (4)	9.20
	Minoxidil (470 μM) + A-23187 (1 μM)	1.06 ± 0.180 (4)	3.39 ± 0.379 (4)	13.0

* Cells (1×10^5) were incubated for 22 hr and the conditioned media, after centrifugation, were analyzed by RIA.
† Values represent the mean ± SD for the number of culture dishes in parentheses.
‡ Where only the mean value is recorded, several appropriate dishes were pooled and the RIA was performed in duplicate. The values agreed within 20% of this mean.

Table 4. Effect of minoxidil on TPA stimulated arachidonic acid metabolism by bovine aorta endothelial cells, bovine aorta smooth muscle cells and porcine aorta endothelial cells*

Cell	Incubation conditions	6-keto-$PGF_{1\alpha}$	PGE_2	$PGF_{2\alpha}$ (ng/ml)	TxB_2
Bovine endothelial	MEM	4.03 ± 1.740 (10)	0.14 ± 0.036 (5)	ND	ND
	Minoxidil (235 μM)	0.71 ± 0.255† (10)	0.34 ± 0.094‡ (5)	ND	ND
	TPA (8.4 nM)	16.02 ± 5.884 (10)	0.34 ± 0.061 (5)	ND	0.17 ± 0.026 (5)
	TPA (8.4 nM) and minoxidil (235 μM)	1.17 ± 0.215§ (10)	0.68 ± 0.013§ (5)	ND	0.40 ± 0.070§ (5)
Bovine smooth muscle	MEM	0.56 ± 0.218 (10)	ND	0.01 ± 0.001 (5)	ND
	Minoxidil (235 μM)	0.29 ± 0.073† (10)	ND	0.02 ± 0.002‖ (5)	ND
	TPA (8.4 nM)	27.08 ± 5.007 (10)	ND	0.07 ± 0.007 (5)	0.07 ± 0.098 (5)
	TPA (8.4 nM) and minoxidil (235 μM)	3.84 ± 0.715§ (10)	ND	0.53 ± 0.059§ (5)	0.31 ± 0.020§ (5)
Porcine endothelial	MEM	0.28 ± 0.071 (8)	0.10 ± 0.042 (8)	ND	ND
	Minoxidil (235 μM)	0.22 ± 0.058‖ (9)	0.54 ± 0.192† (9)	ND	ND
	TPA (1.7 nM)	4.42 ± 1.059 (10)	0.26 ± 0.065 (10)	3.61 ± 0.891 (10)	ND
	TPA (1.7 nM) and minoxidil (235 μM)	0.41 ± 0.079§ (10)	0.40 ± 0.042§ (10)	4.19 ± 0.645‖ (10)	ND

* Cells (1×10^5) were incubated for 22 hr and the conditioned media, after centrifugation, were analyzed by RIA. Data represent the mean values ± SD for the number of culture dishes in parentheses. ND = not determined.
† $P < 0.001$ vs values with MEM. ‡ $P < 0.005$ vs values with MEM. § $P < 0.001$ vs values with TPA. ‖ Not significant.

Table 5. Effect of minoxidil on arachidonic acid metabolism by rat liver cells (the C-9 cell line)*

Reagent	Minoxidil (μM)	6-keto-$PGF_{1\alpha}$ (ng/ml)	$PGF_{2\alpha}$ (ng/ml)
MEM	—	0.16†	
MEM	470	0.11†	
10% Fetal calf serum	—	1.71 ± 0.283 (10)	0.04 ± 0.007 (10)
10% Fetal calf serum	470	0.64 ± 0.169‡ (10)	0.06 ± 0.013§ (10)
A-23187, 0.2 μM	—	1.39 ± 0.189 (10)	0.02 ± 0.007 (10)
A-23187, 0.2 μM	470	0.73 ± 0.151‡ (10)	0.03 ± 0.008§ (10)
TPA, 0.017 μM	—	0.74 ± 0.107 (6)	0.02†
TPA, 0.017 μM	470	0.44 ± 0.049‡ (7)	0.03†
Palytoxin, 11 pM	—	1.21 ± 0.260 (6)	0.03 ± 0.006 (6)
Palytoxin, 11 pM	470	0.68 ± 0.068‡ (7)	0.04 ± 0.005‖ (7)
EGF, 0.018 μM	—	0.33†	ND
EGF, 0.018 μM	470	0.20†	ND

* Cells (4×10^5) were incubated for 22 hr and the conditioned media, after centrifugation, were analyzed by RIA. Data represent the means ± SD for the number of culture dishes shown in parentheses. ND = not determined.

† Where only the mean value is recorded, analyses on duplicate dishes were performed in duplicate. The values agreed within 20% of the means.

‡ $P < 0.001$ vs values with agonist.

§ $P < 0.01$ vs values with agonist.

‖ $P < 0.05$ vs values with agonist.

production by these keratinocytes, after stimulation by recombinant epidermal growth factor; the tumor promoter, TPA, and the Ca^{2+}-ionophore, A-23187, were not reproducibly affected by minoxidil.

The simplest explanation for the inhibition of PGI_2 production but not that of the other cyclooxygenase products by minoxidil was that prostacyclin synthase, not deesterification, was being blocked. Thus, a similar selectivity of inhibition should be found after exogenous substrate is presented to the cells. As expected, only 6-keto-$PGF_{1\alpha}$ production was inhibited by minoxidil when the cells were incubated with 3 μM arachidonic acid for 60 min (Table 6). The levels of the other cyclooxygenase products measured (PGE_2 and/or $PGF_{2\alpha}$) either were not inhibited or were increased slightly.

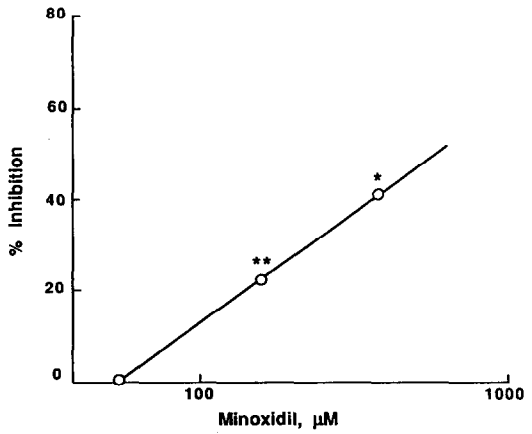

Fig. 6. Inhibition of 6-keto-$PGF_{1\alpha}$ production (rat liver cells, the C-9 cell line, incubated for 20 hr) by increments of minoxidil stimulated with MEM containing 10% fetal calf serum. Data points represent the mean values for ten culture dishes. Key: (**)$P < 0.01$, and (*)$P < 0.001$.

DISCUSSION

Minoxidil selectively inhibited 6-keto-$PGF_{1\alpha}$ production in two endothelial cell lines originating from bovine aorta and porcine aorta endothelial cells, bovine aorta smooth muscle cells, and, although less effectively, rat liver cells. The less effective inhibition of PGI_2 production in the rat liver cells could reflect a slightly different structure of the prostacyclin synthase in the rat liver cells, i.e. the prostacyclin syntheses are either species-specific or tissue-specific. Smith *et al.* [30] have shown by measurements with monoclonal antibodies and immunoradiometric procedures that the prostacyclin synthase enzyme does not differ among species; however, a small change in conformation may not have been detected. The effect of minoxidil on prostacyclin synthase also may be indirect and may depend on penetration of the membrane whose composition varies among cells and species.

The possibility that minoxidil was stabilizing PGI_2 and thus preventing hydrolysis to 6-keto-$PGF_{1\alpha}$, the product being measured by the anti- 6-keto-$PGF_{1\alpha}$ in the RIA, was considered unlikely since the minoxidil required to inhibit the 6-keto-$PGF_{1\alpha}$ produced by the rat liver cells was thirty-five times greater than that required to inhibit 6-keto-$PGF_{1\alpha}$ formation by the bovine endothelial cells (IC_{50} is 700 μM for rat liver cells and 20 μM for bovine endothelial cells). We also were concerned that minoxidil might stimulate the activity of the enzyme 9-hydroxydehydrogenase, which catalyzes the formation of 6-keto-PGE_1 [27]. The 9-hydroxydehydrogenase is present

Table 6. Effect of minoxidil on metabolism of added arachidonic acid by bovine endothelial cells, porcine endothelial cells, bovine smooth muscle cells, rat liver cells and rat keratinocytes*

Cell	Incubation conditions	Arachidonic acid metabolites (ng/ml) 6-keto-$PGF_{1\alpha}$	$PGF_{2\alpha}$	PGE_2
Bovine endothelial	MEM	5.76 ± 1.447 (4)	ND	ND
	Minoxidil (235 μM)	1.12 ± 0.292 (4)	ND	ND
	AA (3 μM)	181.25 ± 26.575 (4)	0.56	1.08
	AA (3 μM) and minoxidil (235 μM)	19.38 ± 3.307 (4)	2.60	2.16
Porcine endothelial	MEM	1.51 ± 0.297 (10)	ND	ND
	Minoxidil (235 μM)	1.34 ± 0.426† (5)	ND	ND
	AA (3 μM)	5.47 ± 0.888 (12)	0.91 ± 0.121 (6)	0.33 ± 0.031 (6)
	AA (3 μM) and minoxidil (235 μM)	1.20 ± 0.421‡ (12)	1.12 ± 0.483§ (6)	0.60 ± 0.042‡ (6)
Bovine smooth muscle	MEM	0.62 ± 0.106 (4)	ND	ND
	Minoxidil (235 μM)	0.19 ± 0.051 (4)	ND	ND
	AA (3 μM)	3.90 ± 0.231 (4)	0.021	ND
	AA (3 μM) and minoxidil (235 μM)	1.11 ± 0.208 (4)	0.101	ND
Rat liver	MEM	ND	ND	ND
	Minoxidil (940 μM)‖	ND	ND	ND
	AA (3 μM)	6.62 ± 0.523 (6)	0.04	0.05
	AA (3 μM) and minoxidil (940 μM)‖	3.25 ± 0.414‡ (6)	0.08	0.30
Rat keratinocytes	MEM		0.16 ± 0.061 (6)	0.66 ± 0.222 (6)
	Minoxidil (470 μM)		0.24 ± 0.069† (6)	1.47 ± 0.545¶ (6)
	AA (3 μM)		1.34 ± 0.139 (6)	7.80 ± 0.358 (6)
	AA (3 μM) and minoxidil (470 μM)		1.09 ± 0.167§ (6)	7.40 ± 0.518§ (6)

* Cells were incubated for 60 min and the conditioned media, after centrifugation, were analyzed by RIA. Cell densities were bovine endothelial, rat liver and rat keratinocytes, 4×10^5/35 mm dish, and porcine endothelial and bovine smooth muscle, 1×10^5/35 mm dish. Data represent mean values ± SD for the number of culture dishes in parentheses. ND = not determined.

† Not significant vs values with MEM.

‡ $P < 0.001$ vs values with AA.

§ Not significant vs values with AA.

‖ Minoxidil at 940 μM did not affect the morphology of the rat liver cells when examined microscopically.

¶ $P < 0.01$ vs values with MEM.

in such diverse tissues as lung, platelets, and endothelial cells [28], and 6-keto-PGE_1 has been shown to have potent biological activity as a vasodilator, inhibitor of platelet aggregation, and promotor of fibrinolysis [28]. Its role in these processes *in vivo* is incompletely understood. Flavone drugs (rutin and naringinum) have been shown to inhibit the conversion of PGI_2 to 6-keto-PGE_1 by 9-hydroxydehydrogenase [28]. Stimulation of the conversion of PGI_2 to 6-keto-PGE_1 would be perceived as a decrease in 6-keto-$PGF_{1\alpha}$ production when measured serologically. The addition of rutin to cultures with minoxidil and A-23187 did not affect the inhibition of 6-keto-$PGF_{1\alpha}$ production.

The relevance of the statistically significant increases in the levels of PGE_2, $PGF_{2\alpha}$ and/or TxB_2 after minoxidil treatment is unknown. These increases do not approach the levels of the inhibited PGI_2. The reasons for this disparity may be very complex, but it would appear that the synthesized PGH_2 is at or close to saturation for the PGE_2 isomerases, TxA_2 synthases and/or $PGF_{2\alpha}$ reductases. The possibility that minoxidil is also inhibiting synthesis of the endoperoxide cannot be ruled out. The nature of the decomposition products of the unstable endoperoxide remaining after minoxidil's inhibition is not known. Such endoperoxide products may not have been secreted into the conditioned media.

The pharmacological effect we observed may not be relevant to the anti-hypertensive properties of minoxidil. PGI_2 has been known for some time to have vasodilator properties [31]. It therefore seems paradoxical that a drug with known vasodilator properties would have the effect of inhibiting PGI_2 production. Nevertheless, it must be kept in mind that neither the mechanisms by which PGI_2 relaxes smooth muscle nor the interactions of endothelium-smooth muscle in the vasodilatory response are well understood. The effects seen in our studies were apparent using doses in the micromolar range, whereas doses of the drug in humans that decrease arterial pressure give peak serum levels in the range of 0.7 μM, at least 10-fold less. Most likely arachidonic acid metabolism is not causally related to the anti-hypertensive properties of minoxidil. If inhibition of PGI_2 production were solely related to the anti-hypertensive properties of minoxidil, non-steroidal anti-inflammatory drugs, which also inhibit PGI_2 synthesis, would have similar anti-hypertensive properties.

The relationship between the effect of minoxidil

on arachidonic acid metabolism and increased growth of vellus hair [5, 6], if any, is not clear. It is possible that arachidonic acid metabolites of the capillary endothelial cells in proximity to the keratinocytes are affecting keratinocyte function by a paracrine-stimulating mechanism. In this regard, the effects of other drugs that increase growth of vellus hair on arachidonic acid metabolism would be of interest. A number of biological and biochemical actions have now been attributed to minoxidil, a drug which also has wide-ranging physiologic effects. Among the biological and biochemical events described are delayed senescence of keratinocytes, suppression of lymphocyte activity, inhibition of lysylhydroxylase and, now, inhibition of prostacyclin synthase. All of these effects have been observed at concentrations higher than those achieved with oral administration in standard doses. The tissue concentration of the drug when applied topically has not been measured. If local levels of minoxidil approach those shown to inhibit PGI_2 production, any beneficial actions of PGI_2 may be adversely affected.

No unifying hypothesis can be formed to account for the effects of minoxidil by one biochemical action, and it is possible that the stimulation by minoxidil of growth of vellus hair is due to actions of the drug different from those responsible for hypotensive activity seen after oral administration.

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REFERENCES

1. H. J. Dargie, C. T. Dollery and J. Daniel, *Lancet* **2**, 515 (1977).
2. B. L. Devine, R. Fife and P. M. Trust, *Br. med. J.* **2**, 667 (1977).
3. D. W. DuCharme, W. A. Freyburger, B. E. Graham and R. G. Carlson, *J. Pharmac. exp. Ther.* **184**, 662 (1973).
4. E. G. Spokas, G. Folco, J. Quilley, P. Chander and J. C. McGiff, *Hypertension* **5**, (Suppl. I), I-107 (1983).
5. J. L. Burton and A. Marshall, *Br. J. Derm.* **101**, 593 (1979).
6. R. N. Earhart, J. Ball, D. D. Nuss and J. L. Aeling, *Sth. med. J.* **70**, 442 (1977).
7. A. J. Mitchell and E. A. Krull, *J. Am. Acad. Derm.* **11**, 763 (1984).
8. M. Seidman, M. Westfried, R. Maxey, T. K. Rao and E. A. Friedman, *Cutis* **28**, 551 (1981).
9. V. C. Weiss, D. P. West and C. E. Mueller, *J. Am. Acad. Derm.* **5**, 224 (1981).
10. V. C. Weiss, D. P. West, T. S. Fu, L. A. Robinson, B. Cook, R. L. Cohen and D. A. Chambers, *Archs Derm.* **120**, 457 (1984).
11. H. P. Baden and J. Kubilus, *J. invest. Derm.* **81**, 558 (1983).
12. R. L. Cohen, M. E. Alves, V. C. Weiss, D. P. West and D. A. Chambers, *J. invest. Derm.* **82**, 90 (1984).
13. G. M. P. Gailbraith and B. H. Thiers, *Int. J. Derm.* **24**, 249 (1985).
14. S. Murad, J. Clayton and S. R. Pennell, *J. invest. Derm.* **86**, 496 (1986).
15. L. M. Alderson, G. Endemann, S. Lindsey, A. Pronczuk, R. L. Hoover and K. C. Hayes, *Am. J. Path.* **123**, 334 (1986).
16. F. M. Booyse, B. J. Sedlak and M. E. Rafelson, *Thromb. Diath. haemorrh.* **34**, 825 (1975).
17. E. A. Jaffe, L. W. Hoyer and R. Nachman, *J. clin. Invest.* **52**, 2757 (1973).
18. H. P. Baden and J. Kubilus, *J. invest. Derm.* **80**, 124 (1983).
19. S. R. Coughlin, M. A. Moskowitz, B. R. Zetter, H. N. Antoniades and L. Levine, *Nature, Lond.* **288**, 600 (1980).
20. S. L. Hong, C. M. Wheless and L. Levine, *Prostaglandins* **13**, 271 (1977).
21. A. Rigas and L. Levine, *J. Pharmac. exp. ther.* **231**, 230 (1984).
22. H. J. Phillips, in *Tissue Culture Methods and Applications* (Eds. P. F. Kruze, Jr. and M. K. Patterson, Jr.), pp. 406–8. Academic Press, New York (1973).
23. L. Levine, in *Manual of Clinical Laboratory Immunology* (Eds. J. L. Fahey, H. Friedman and N. R. Rose), 3rd Edn, p. 685. American Society for Microbiology, Washington, DC (1986).
24. L. Levine and T. Kobayashi, *Prost. Leuk. Med.* **12**, 357 (1983).
25. I. Alam, K. Ohuchi and L. Levine, *Analyt. Biochem.* **93**, 339 (1979).
26. P. K. Moore, R. J. Griffiths and F. J. Lofts, *Biochem. Pharmac.* **32**, 2813 (1983).
27. J. C. McGiff, *A. Rev. Pharmac. Toxic.* **21**, 479 (1981).
28. J. R. S. Hoult and R. K. Moore, *Trends pharmac. Sci.* **7**, 197 (1986).
29. J. D. Kvedar and L. Levine, *J. invest. Derm.* **88**, 124 (1987).
30. W. L. Smith, D. L. DeWitt and J. S. Day, in *Advances in Prostaglandin, Thromboxane, and Leukotriene Research* (Eds. B. Samuelsson, R. Paoletti and P. Ramwell), Vol. 11, p. 87. Raven Press, New York (1983).
31. S. Moncada and J. R. Vane, *Pharmac. Rev.* **30**, 293 (1979).