(*19*). Furthermore, during the 10-year period from 1963 to 1972 when aerosol production in the United States nearly tripled, the output of spray packaging in the free world outside the United States almost quadrupled (*19*), an indication of the higher growth rate of aerosol production outside the United States. Data on FCC production outside the free world are not available, but the quantity produced is estimated to be relatively low. From this information and the review given above of the relationship between applications and environmental release, it is apparent that annual FCC losses from sources outside the United States are presently approximately equal to and in the future will probably exceed U.S. losses. More detailed conclusions from the available data do not seem warranted. However, even from these qualitative estimates, it would appear that restrictions on FCC production and use in the United States would only partly reduce, rather than end, destruction of stratospheric O_3, if, in fact, the chlorine-catalyzed O_3 destruction due to FCC compounds can exceed all natural sinks of stratospheric O_3.

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Angiotensin II Stimulation of Prostaglandin Production in Cultured Human Vascular Endothelium

Abstract. *Immunoreactive material resembling prostaglandin E accumulates in the medium of cultured human umbilical vein endothelial cells. Production is inhibited by indomethacin and stimulated by angiotensin II. Prostaglandin secretion by endothelium may be important in platelet-dependent thrombotic phenomena, and in local control of vascular permeability and tone in vivo.*

Exogenous prostaglandins (PG's) are potent mediators of several cardiovascular phenomena (*1*). In particular, prostaglandin E (PGE) can induce changes in vascular tone and permeability (*2*), and modify the behavior of blood platelets (*3*). Little is known, however, about the specific origins of endogenous PG's or the physiologic factors controlling their biosynthesis within the circulatory system. Recent work in our laboratory (*4*) and by others has led to the culture of homogeneous populations of vascular endothelial cells for biochemical and morphological studies. We now report the secretion of immunoreactive PGE-like material (iPGE) by cultured human endothelium and stimulation of basal levels of production by the vasoactive octapeptide angiotensin II.

Human endothelial cells were obtained, free of contamination by vascular smooth muscle, fibroblasts, and circulating blood elements, by brief collagenase treatment of the luminal surface of term umbilical cord veins (*4*). Cells from each vein were plated in replicate in 12 to 24 Linbro multidish culture wells and were allowed to grow to confluent densities (1 to 2×10^5 cells per square centimeter) in medium 199 supplemented with fetal bovine serum (20 percent) at 37°C under a humidified atmosphere consisting of 5 percent CO_2 in air. Primary cultures obtained in this manner form uniform epithelioid monolayers that exhibit differentiated ultrastructural characteristics of vascular endothelium. All experiments were performed on 5- to 10-day-old confluent cultures.

The concentration of PGE-like material was determined on frozen samples of culture medium or on samples of sonicated cells by a specific radioimmunoassay (*5*). After acidification to *p*H 3.5 and extraction with ethyl acetate, prostaglandin E was separated by silicic acid chromatography and converted to prostaglandin B by treatment with 0.1*N* methanolic potassium hydroxide. Radioimmunoassay was performed with sheep antiserum to PGA_1. At a final dilution of 1 : 75,000 it bound [^{3}H]PGB, and gave a standard curve ranging from 0.08 to 2.0 ng of PGB_2. Fifty percent inhibition of binding for PGB_2, PGB_1, PGE_2, PGE_1, and $PGF_{2\alpha}$ occurred with 0.5, 0.09, 12.0, 5.5, and >100 ng, respectively. Recoveries of known amounts of PGE_2, ranging from 0.8 to 5.6 ng, added to plasma and handled in this manner were 104 ± 19.8 percent (mean ± 1 S.D.), with a mean absolute error of 0.22 ng over the entire range. This assay procedure measures both PGE_1 and PGE_2, which we refer to as iPGE.

Culture medium incubated with endothelial cells for 24 hours contained 13.6 ± 1.5 ng of iPGE per milliliter (mean ± S.E.M.; six cultures), while washed, rapidly frozen cells from these cultures that were sonicated and suspended in phosphate-buffered saline (in a volume equal to that of the culture medium) yielded 1.35 ± 0.11 ng of iPGE per milliliter. Fresh culture medium and medium incubated in the absence of cells contained less than 0.2 ng of iPGE per milliliter.

To establish that the extracellular accumulation of iPGE reflected new synthesis, rather than release of stored material, indomethacin, a potent, selective inhibitor of prostaglandin biosynthesis (*6*), was added to the culture medium at the start of the 24-hour incubation. A dose-related reduction in iPGE production resulted (Fig. 1). Fifty percent inhibition occurred at 6×10^{-9} g per milliliter of indomethacin. Cytotoxic changes were not observed by phase-contrast microscopy in cultures exposed to indomethacin at any of the concentrations tested. These data indicate that the appearance of iPGE in the medium was the result of de novo synthesis by the cultured endothelial cells.

Robertson and Khairallah have demonstrated endothelial cell contraction and transient permeability changes in the walls

of blood vessels in response to angiotensin II (*7*). These observations indicate a new biological activity for this vasoactive octapeptide, distinct from its role in the control of circulatory peripheral resistance. In these studies, exogenous PGE also produced similar changes in vascular endothelium. Since endogenous prostaglandins are generally thought to act locally, in or near the tissue in which they are formed (*8*), the observed effects of angiotensin II may have been mediated through stimulation of PGE production in vascular endothelial cells. We, therefore, studied the influence of angiotensin II on iPGE secretion by cultured endothelium. To minimize enzymatic degradation of added angiotensin II or secreted prostaglandins, and to avoid the contribution of unknown vasoactive substances by serum, these experiments were performed in serum-free, chemically defined medium. Confluent primary endothelial cultures (10^5 cells per well) were rinsed gently with phosphate-buffered saline and reincubated with serum-free medium 199 for 2 hours at 37°C. Basal iPGE secretion into the culture medium, under these conditions, averaged 0.009 ± .002 ng per microgram of cell protein per 2 hours (mean ± S.E.M.; three experiments). Addition of angiotensin II (Hypertensin-Ciba) to cultures plated in replicate at 0.05×10^{-7} to $5.0 \times 10^{-7} M$ concentrations produced a dose-related stimulation of iPGE production (Fig. 2). At maximal stimulation (1.0×10^{-7} to $5.0 \times 10^{-7} M$ angiotensin II) the amount of iPGE in the culture medium was approximately 100 times greater than that of the intracellular content of unstimulated cells. Therefore, angiotensin II appears to act by increasing the de novo synthesis of iPGE, rather than its passive release.

Angiotensin II has been shown to stimulate the release of PGE-like material into the venous effluent of various isolated, perfused organs and tissues (*9, 10*). In certain of these studies, the vasculature per se was postulated as the source of PGE synthesis, but the precise cellular origin or origins could not be determined in the experimental models used (*10*). By isolating homogeneous populations of human vascular endothelium in culture, we have identified this cell type as a potential source of PGE secretion in response to angiotensin II. This method also should prove useful in characterizing the spectrum of prostaglandins produced by endothelium, and the influence of other vasoactive mediators, such as histamine, serotonin, bradykinin, and catecholamines, on their biosynthesis.

Both PGE_1 and PGE_2 cause an increase in small vessel permeability and induce vasodilatation (*2*). PGE_1 is also chemotactic for polymorphonuclear leukocytes, and PGE_2 accumulates in inflammatory exudates (*11*). PGE_2 stimulates the second phase of human platelet aggregation, while PGE_1 inhibits platelet aggregation induced by adenosine diphosphate and suppresses platelet thrombus formation in traumatized vessels (*3*). The endothelial cell is the principal structural component of small blood vessels, and, as such, is intimately involved in each of these processes, functioning as both permeability barrier and nonthrombogenic surface. Thus, pathophysiologic factors that modulate secretion of PGE by this cell may be important in the local control of vascular tone and permeability and platelet-dependent thrombotic phenomena.

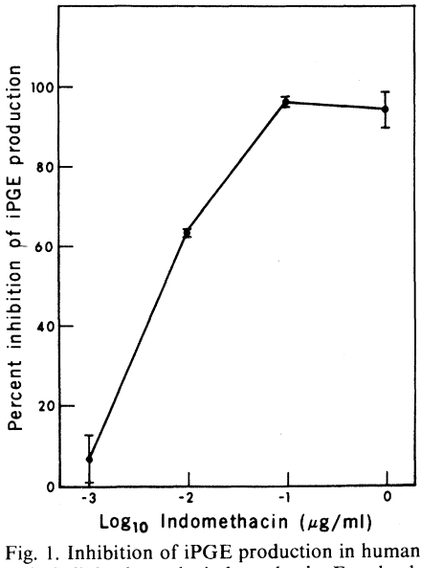

Fig. 1. Inhibition of iPGE production in human endothelial cultures by indomethacin. Equal volumes of fresh medium containing no additions, or the indicated concentrations of indomethacin, were added to triplicate confluent cultures in each experiment. After the cultures were incubated at 37°C for 24 hours, the concentration of iPGE in the culture medium was determined by radioimmunoassay, and related to the protein content of the washed, sonicated cells in each culture well. Untreated cultures produced 0.129 ± .017 ng of iPGE per microgram of cell protein per 24 hours (mean ± S.E.M.). Brackets indicate the S.E.M. of mean values in two separate experiments.

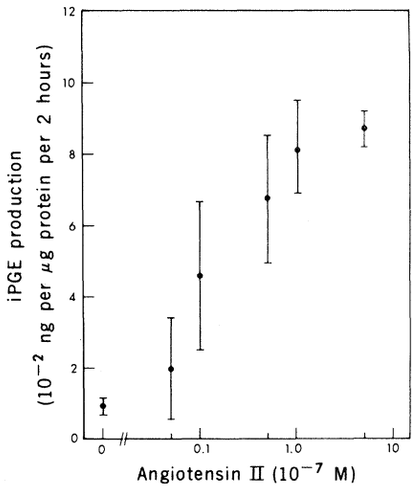

Fig. 2. Stimulation of iPGE production in human endothelial cultures by angiotensin II. Angiotensin II (Hypertensin-Ciba), freshly prepared in serum-free medium 199, contained negligible levels of iPGE before and after incubation in the absence of cells for 2 hours at 37°C. In each experiment duplicate confluent cultures were incubated with equal volumes of serum-free medium, or medium containing the indicated concentrations of angiotensin II, for 2 hours at 37°C. The concentration of iPGE in the culture medium was then determined and related to the protein content of the washed, sonicated cells from each culture well. Brackets indicate the S.E.M. of mean values in three separate experiments.

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