Angiotensin II Stimulation of Vascular Smooth Muscle Phosphoinositide Metabolism

State of the Art Lecture

KATHY K. GRIENDLING, BRADFORD C. BERK, PETER GANZ, MICHAEL A. GIMBRONE, JR., AND R. WAYNE ALEXANDER

SUMMARY Phosphoinositide hydrolysis is an integral step in the activation of vascular smooth muscle by angiotensin II. Sequential phospholipase C-mediated hydrolysis of the polyphosphoinositides and phosphatidylinositol in cultured vascular smooth muscle cells stimulated with angiotensin II results in a coordinated series of biochemical events: a transient formation of inositol trisphosphate associated with calcium mobilization, and a biphasic, sustained formation of diacylglycerol associated with activation of protein kinase C and cytosolic alkalization. The initial, rapid phase and the sustained phase of the angiotensin II response appear to be differentially controlled. Formation of inositol trisphosphate and mobilization of calcium are attenuated by activation of protein kinase C. Sustained diacylglycerol formation is promoted by cytosolic alkalization, and appears to require cellular processing of the angiotensin II-receptor complex. Calcium and cyclic guanosine 3',5'-monophosphate do not appear to regulate phospholipase C-mediated phosphoinositide hydrolysis in vascular smooth muscle. Thus, regulation of angiotensin II-stimulated second messenger generation in vascular smooth muscle is complex, perhaps involving protein kinase C activation, changes in intracellular pH, and processing of the angiotensin II-receptor complex.

(Hypertension 9 [Suppl III]: III-181-III-185, 1987)

KEY WORDS  • diacylglycerol • inositol trisphosphate • sodium-hydrogen exchange

CALCIUM-MOBILIZING hormones have been shown to stimulate phosphoinositide metabolism in a variety of cell types.1-4 This agonist-induced, phospholipase C (PLC)–mediated hydrolysis of the phosphoinositides results in the generation of two putative second messengers, inositol trisphosphate (IP₃) and diacylglycerol (DG). Each of these molecules serves a different function within the cell: IP₃ mediates calcium mobilization from the endoplasmic reticulum, and DG activates the calcium- and phospholipid-sensitive enzyme, protein kinase C.

In cultured vascular smooth muscle cells (VSMCs) stimulated with angiotensin II (ANG II), formation of IP₃ and DG have been clearly demonstrated.5-8 The mechanisms controlling generation of these two second messengers have only been partially elucidated, and their separate and synergistic biochemical and physiological consequences are not fully understood. We review here recent data on the role of phosphoinositide metabolism in ANG II activation of vascular smooth muscle, and discuss the various possible mechanisms controlling the agonist-mediated phosphoinositide response in VSMCs.

Phosphoinositides and ANG II Activation of Vascular Smooth Muscle

ANG II causes a rapid contraction of rat aorta that attenuates significantly after several minutes even in the continued presence of agonist9 (Figure 1A). Attenuation of the contractile response is temporally associated with a fall in phosphatidic acid,10 a metabolite of DG, suggesting that sustained contraction might be dependent upon continued formation of DG. To facilitate the study of cellular and biochemical events associated with ANG II–mediated activation of vascular smooth muscle, we have performed experiments using

From the Cardiovascular Division (K. K. Griendling, B. C. Berk, P. Ganz, R. W. Alexander), Department of Medicine and Vascular Research Division, Department of Pathology (M. A. Gimbrone, Jr.), Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts. Supported by NIH Grants HL 706321, HL 34874, and HL 22602.

Address for reprints: Kathy K. Griendling, Ph.D., Cardiovascular Division, Brigham and Women’s Hospital, 75 Francis Street, Boston, MA 02115.
 activation of vascular smooth muscle by ANG II (100 nM). A. Time course of ANG II-stimulated contraction of helical strips of rat aorta. Force development is expressed as a percentage of the maximum increase. 9 B. Time course of ANG II-stimulated changes in cytosolic free calcium ($\text{Ca}^{2+}$), measured by quin 2 fluorescence in cultured rat aortic vascular smooth muscle cells (VSMCs). 5 C. Time course of ANG II-stimulated formation of diacylglycerol (DG) and inositol trisphosphate (IP$_3$) in cultured VSMCs, expressed as a percentage of the unstimulated level of DG (2,608 cpm) or IP$_3$ (1,015 cpm). 5, 6 D. Time course of ANG II-stimulated changes in intracellular pH measured by 2',7'-bis-(2-carboxyethyl)-5(and -6)carboxyfluorescein in cultured rat aortic VSMCs. 22
tion then would continue, with PI as the source. Activation of protein kinase C may selectively inhibit the early events in ANG II stimulation of VSMCs, and therefore serve as a control mechanism to modulate the early phosphoinositide response.

Calcium

Since the increase in [Ca\(^{2+}\)], is one of the earliest events resulting from activation of many cells by non-adenylate cyclase-coupled hormones, it has been suggested that calcium itself may serve to regulate the course of phosphoinositide metabolism.\(^{19}\) In other systems, several forms of PLC have been isolated with different pH and calcium concentration optima for enzyme activity.\(^{20}\) Furthermore, in isolated unilamellar vesicles, PI hydrolysis by purified PLC has been shown to be calcium-dependent.\(^{21}\) These observations suggest that the increase in [Ca\(^{2+}\)] resulting from ANG II stimulation of IP\(_3\), formation may serve to shift dominant PLC activity from the polyphosphoinositides to PI. However, this does not appear to be the case in VSMCs, since the calcium ionophore ionomycin (15 \(\mu\)M) does not cause breakdown of PI.\(^{6}\) Thus, in VSMCs, calcium alone cannot mimic the hormonal response and therefore is not likely to be responsible for induction of the delayed phosphatidylinositol hydrolysis. It remains possible, however, that calcium modulates the activity of other enzymes involved in phosphoinositide metabolism.

Intracellular pH

The intracellular concentration of hydrogen ion (pH) has also been postulated to be important to the function of enzymes involved in the phosphoinositide pathway. Activity of purified PLC has recently been shown to have a strong pH dependency. Naknishi et al.\(^{22}\) have isolated two forms of PLC with different activities at alkaline or acidic pH, depending upon calcium concentration. This putative control mechanism may be particularly relevant in VSMCs, since ANG II alters pH, in VSMC grown on cover slips.\(^{22}\) Starting from a resting pH of 7.15 to 7.35, ANG II increases rapidly within the cell in response to ANG II, generally over a period of minutes,\(^{30,31}\) it seems probable that early acidification is attributable to calcium-activated H\(^+\) transport; that is, when [Ca\(^{2+}\)] increases rapidly within the cell in response to ANG II, some of it is electroneutrally extruded, perhaps via the plasma membrane Ca\(^{2+}\)-adenosine triphosphatase (ATPase), in exchange for hydrogen (Berk et al., unpublished observations). Subsequent alkalinization appears to be the result of a stimulation of Na\(^+\)-H\(^+\) exchange, since it can be inhibited by dimethylamiloride.\(^{22}\) Whether such changes in pH serve to modulate DG and IP\(_3\), formation is at present unclear. Alkalinizing VSMCs with ammonium chloride enhances late ANG II-stimulated DG formation, while acidifying the cells with potassium acetate inhibits this response (unpublished observations). Neither intervention has much effect on the early DG/IP\(_3\), response, suggesting that the intracellular alkalinization following ANG II stimulation of VSMCs may be important in the delayed induction of PI hydrolysis rather than in the attenuation of early polyphosphoinositide hydrolysis. Activation of protein kinase C by DG produced in response to ANG II may serve not only to attenuate PIP\(_2\) hydrolysis (see above), but also to alkalinize the cell via receptor-coupled Na\(^+\)-H\(^+\) exchange, thereby promoting sustained DG formation.

Cyclic GMP

Cyclic guanosine 3',5'-monophosphate (cGMP), a mediator of vascular smooth muscle relaxation\(^{23}\) and an inhibitor of vessel contraction, has been proposed as an inhibitor of PI hydrolysis in platelets.\(^{24}\) It is thus possible that cGMP mediates vascular smooth muscle relaxation by attenuating hormonally induced phosphoinositide metabolism. Rapoport\(^{25}\) has shown that 8-bromo-cGMP inhibits both norepinephrine-induced contraction and inositol monophosphate generation in rat thoracic aorta. Furthermore, Ganz et al.\(^{26}\) have demonstrated that cGMP moderately attenuates both early and late ANG II-induced DG formation in cultured rat aortic VSMCs. However, this inhibition is probably not due to inhibition of PLC, since PIP\(_2\) hydrolysis and IP\(_3\) formation are not similarly attenuated (unpublished observations). Thus, although cGMP may modulate DG accumulation in VSMC, it does not appear to inhibit PLC-mediated PI/IP\(_3\), hydrolysis.

Cellular Processing of the ANG II-Receptor Complex

Recent work has shown that \(^{125}\)I-ANG II bound to adrenal glomerulosa and adrenocortical cells is internalized via endocytosis,\(^{27,28}\) and can be demonstrated in lysosomes in less than 20 minutes.\(^{27}\) In vascular smooth muscle, binding of ANG II has been shown to initiate aggregation and subsequent internalization of the ANG II receptors.\(^{29}\) Since these cellular events generally occur over a period of minutes,\(^{30,31}\) it seems possible that the temporal delay in the onset of PLC-mediated PI breakdown (>2 minutes) observed in ANG II-stimulated VSMCs may be related to some obligatory cellular events involving processing of the agonist-receptor complex. Hokin\(^2\) has demonstrated that the majority of acetycholine-stimulated \(^{32}\)P-ATP incorporation into PI in pigeon pancreatic slices occurs in the endoplasmic reticulum, suggesting that the surface signal must somehow be translocated for PI hydrolysis to occur. There is some evidence to support this hypothesis in VSMCs. Two interventions that interfere with receptor processing, low temperature and phenylarsine oxide, also disrupt development of sustained ANG II-induced DG formation in VSMCs, while only minimally affecting the early phosphoinositide response.\(^{32}\) Decreasing the temperature from 37°C to 19°C, which prevents fusion of endocytotic vesicles with lysosomes,\(^{33}\) slightly delays the early ANG II-stimulated DG peak and dramatically attenuates devel-
Potential mechanisms controlling phosphoinositide hydrolysis in vascular smooth muscle.

A. Interrelationship of biochemical events resulting from stimulation of vascular smooth muscle cells with ANG II. B. Cellular events potentially important in the development of the sustained response. PI = phosphatidylinositol; PIP = phosphatidylinositol 4-phosphate; PIP$_2$ = phosphatidylinositol 4,5-bisphosphate; DG = diacylglycerol; IP = inositol monophosphate; IP$_2$ = inositol bisphosphate; IP$_3$ = inositol trisphosphate; PLC = phospholipase C.

Development of the second, sustained phase of DG formation. Further reduction of temperature (to 4°C) to block receptor internalization, results in a delayed, monophasic formation of DG in response to ANG II, which derives from PIP$_2$ rather than PI (unpublished observations). Phenylarsine oxide, which also blocks ANG II–receptor internalization in these cells, selectively inhibits the sustained phase of ANG II–induced DG formation (81 ± 6% inhibition). Although not conclusive, these experiments suggest that cellular processing of the ANG II–receptor complex may be essential to development of the late DG response, and as such may provide a mechanism for regulation of sustained cellular response.

Conclusions

Activation of VSMCs by ANG II results in the PLC-mediated hydrolysis of PIP$_2$/PIP to form IP$_3$ and DG, and in the subsequent hydrolysis of PI to form DG. These second messengers are then responsible for calcium mobilization, protein kinase C activation, alterations in intracellular pH (perhaps indirectly), and ultimately, contraction. The mechanisms controlling the initial and more prolonged cellular responses to ANG II are at present incompletely understood. The mechanisms that we consider most likely to be important are depicted in Figure 2. Figure 2A describes the biochemical events that occur after ANG II stimulation and depicts possible biochemical processes controlling phosphoinositide metabolism, calcium mobilization, and intracellular alkalization. We suggest that early formation of DG from PIP$_2$ activates protein kinase C, which then feeds back to attenuate further PIP$_2$ hydrolysis, IP$_3$ formation, and calcium mobilization. Activation of protein kinase C may also stimulate Na$^+$–H$^+$ exchange, resulting in intracellular alkalization. The secondary increases in pH$_i$ may regulate the subsequent phosphoinositide response by promoting sustained DG formation. Figure 2B depicts the cellular events that may be involved in regulation of DG formation. Preliminary evidence strongly suggests a role for cellular processing of the ANG II–receptor complex in the sustained DG response. The biochemical events discussed earlier, particularly changes in pH$_i$, may also modulate these cellular events by some as yet undescribed mechanism. It is clear that regulation of ANG II–stimulated second messenger generation in VSMCs is complex. Much work remains to be done before our understanding of the mechanisms controlling phosphoinositide metabolism is complete.

Note added in proof: The unpublished observations of Berk et al. mentioned in the text have now been published.

Acknowledgments

We gratefully acknowledge the secretarial assistance of Ms. Susan McHale and Ms. Paula Dolan. We also are indebted to Drs. Tommy A. Brock and Susan E. Rittenhouse for helpful discussions and expert advice.
References

10. Danthuluri NR, Deth RC. Acute desensitization to angiotensin II: evidence for receptor regulation of agonist-induced diacylglycerol production during tonic contraction of rat aorta. Eur J Pharmacol 1986;126:135–139
14. Gershengorn MC, Imai A. Phosphatidylinositol 4,5-bisphosphate turnover is transient while phosphatidylinositol turnover is persistent in thyrotropin-releasing hormone-stimulated rat pituitary cells. Proc Natl Acad Sci USA 1986;83:8540–8544
28. McCrae A, Penney A, Sarz JM. Processing of angiotensin II (All) and (Sar1, Ala8) All by cultured bovine adrenocortical cells. Endocrinology 1986;118:2312–2318
34. McCrae A, Penney A, Sarz JM. Processing of angiotensin II (All) and (Sar1, Ala8) All by cultured bovine adrenocortical cells. Endocrinology 1986;118:2312–2318
Angiotensin II stimulation of vascular smooth muscle phosphoinositide metabolism.

State of the art lecture.

K K Griendling, B C Berk, P Ganz, M A Gimbrone, Jr and R W Alexander

Hypertension. 1987;9:III181
doi: 10.1161/01.HYP.9.6_Pt_2.III181

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/9/6_Pt_2/III181