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ANGIOTENSIN II AND BRADYKININ STIMULATE PHOSPHOINOSITIDE BREAKDOWN IN INTACT RAT KIDNEY GLOMERULI BUT NOT IN PROXIMAL TUBULES: GLOMERULAR RESPONSE MODULATED BY PHORBOL ESTER

M.Chandra Sekar*, Maria Yang**, Elias Meezan **, and Dennis J. Pillion **

Department of Pharmacology and ** Metabolic Research Laboratory, University of Alabama at Birmingham, UAB Station, Birmingham, AL 35294

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We have investigated the effect of angiotensin II, bradykinin, insulin and insulin-like growth factor I on phosphoinositide turnover in intact rat glomeruli and tubules. Angiotensin II produced a dose-dependent increase in inositol monophosphate formation with an IC_{50} of 10^{-7} M, when added to isolated rat glomeruli. Angiotensin II-stimulated inositol phosphates formation was inhibited by the angiotensin receptor antagonist [Sar-Leu8]angiotensin II, indicating that the above response was mediated through activation of an angiotensin receptor in intact glomeruli. Besides angiotensin, in intact glomeruli, only bradykinin stimulated a phosphoinositide response, while in intact proximal tubules, none of the agonists tested produced an activation of the inositol phosphate formation. Angiotensin II- and bradykinin-stimulated inositol phosphate accumulation in intact glomeruli was inhibited by phorbol myristate acetate, an activator of protein kinase C.

The kidney plays an important role in the maintenance of the volume and composition of body fluids, and therefore its functional response is finely regulated by various hormonal and neural processes. Receptors for angiotensin (1-5), insulin (6-8), IGF-1 (9), IGF-II (10), bradykinin (11), vasopressin 12) and more recently endothelin (13) and interleukin-1 (14) have been demonstrated in different regions of the kidney. Angiotensin II (AII) plays a role in the regulation of glomerular filtration rate, renal plasma flow, and afferent and efferent arteriolar resistance (15). Only recently, Barnett et al (16) demonstrated AII-mediated contraction of intact glomeruli, which was attenuated by dopamine (16). Most studies investigating the molecular mechanism of action of AII in glomeruli have employed cultured mesangial cells (3,4), where the AII receptors are localized.

* Author to whom reprint requests should be addressed.

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To our knowledge, there has been only one report on the AII-stimulated inositol lipid response in intact glomeruli (17). AII stimulation caused a rapid decrease in [^{3}H]glycerol- and [^{32}P]-labeled PIP_2. In this study, we investigated the phosphoinositide response, as measured by [^{3}H]inositol phosphates formation, following AII, bradykinin, insulin, and IGF-1 stimulation in intact rat glomeruli and tubules.

Materials and Methods

Sprague-Dawley rats (250-300g) were obtained from Harlan Co Indiana; IGF-1 was obtained from Amgen; dopamine, angiotensin and insulin were from Sigma, bradykinin from Calbiochem, [^{3}H] inositol [60.6 Ci/mmole] was from New England Nuclear.

For each experiment, glomeruli and tubules from 4-5 rats were isolated following kidney perfusion with magnetic iron oxide particles, as described by Meezan et al., (18). Tissue was incubated with 40 μCi of [^{3}H]-inositol in 3 ml of Krebs-Ringer phosphate (KRP) buffer containing 0.1% BSA for 3 hours. Excess label was removed by washing, followed by incubation of tissue in the presence or absence of the agonist for the required time in KRP buffer containing 0.1% BSA and 10 mM lithium chloride. Incubation was terminated by the addition of 2 mM EDTA to the tissues and immersion of the sample in boiling water for 5 minutes. Inositol phosphates in the 12,000g supernatant were separated by HPLC on a 10 cm Dupont Zorbax anion exchange column. The elution profile consisted of the following steps: 2 min with 100% water; 20 min with a linear gradient of 0 to 20% 1 M ammonium formate (pH 3.75); 25 min with a linear gradient of 20 to 100% 1M ammonium formate. Flow rate throughout the elution profile was 1.2 ml/min. Fractions were counted by liquid scintillation spectroscopy.

Results and Discussion

The dose response relationship of AII-stimulated inositol monophosphate formation in intact glomeruli is shown in Fig 1. Isolated [^{3}H]inositol-labeled intact rat glomeruli were incubated in the presence of various amounts of AII. There was a dose-dependent increase in inositol monophosphate (IP) formation, with 50% of maximal response occuring at 10^{-7}M AII. This value is in excellent agreement with the earlier observation of Ochi et al (17). AII (10^{-6}M) produced a stimulation of inositol monophosphate formation of (2.5 ± 0.9)- fold over control (n=5) in under these experimental conditions. Little [^{3}H]inositol bis- and trisphosphates were formed in glomeruli under these conditions and therefore statistically significant changes in response to AII were difficult to observe (not shown). In an experiment where inositol phosphates formation by

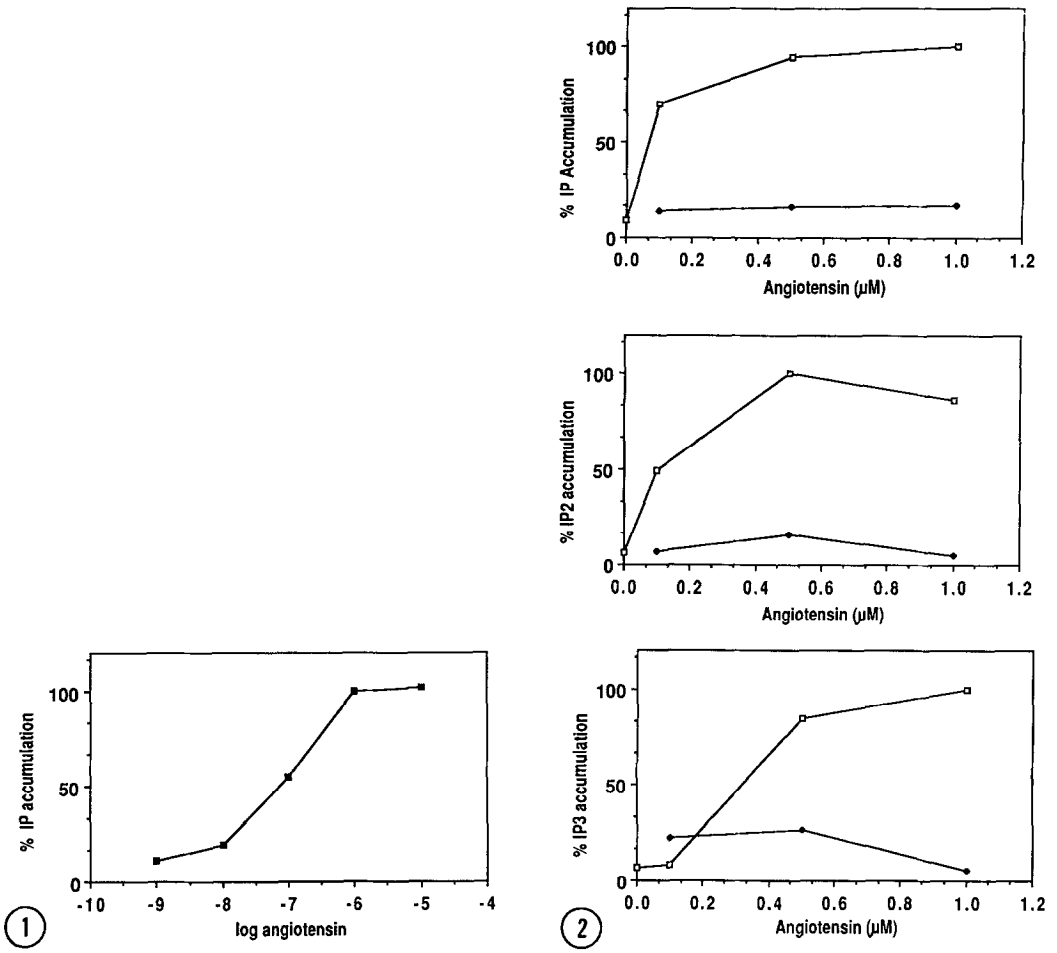


Fig 1. Dose response relationship of AII stimulated inositol monophosphate accumulation in intact glomeruli.

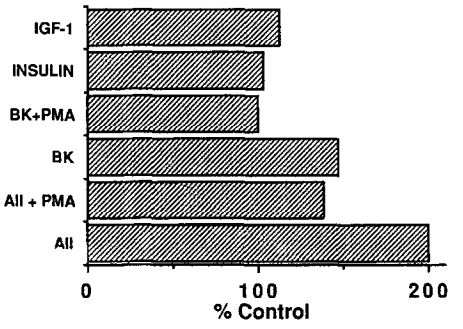
Fig 2. AII-stimulated inositol phosphates accumulation is inhibited by the angiotensin antagonist [sar-leu8]Angiotensin. AII alone (□) ; AII + [sar-leu8]AII (10^{-5}M) (■).

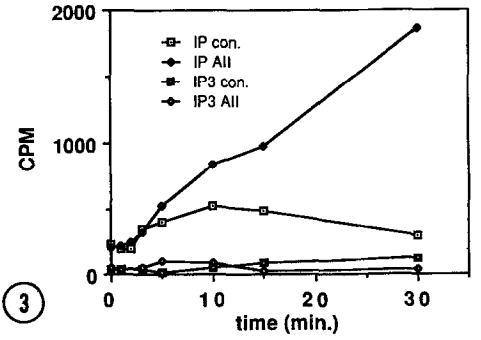
rat glomeruli were optimized to give 3200 cpm at 1μM AII, we did observe a small dose-dependent increase in IP_2 and IP_3 formation; Increases in all three of the inositol phosphates were abolished in the presence of the AII antagonist [Sar-Leu8]AII (fig2). This result indicates that the AII-stimulated inositol phosphates formation by glomeruli is the result of AII receptor activation.

It was possible that more significant and larger increases in inositol polyphosphates formation were occuring at earlier time periods. Time-course studies (fig 3), however, do not show any peak for inositol trisphosphates

formation at earlier time periods. Consistent with the reports in other tissues, significant increases in inositol monophosphate could be observed in isolated glomeruli after 5 minutes of incubation with agonist.

Ochi et al (17) on the other hand did observe a significant decrease in both [^{3}H] glycerol and [^{32}P] PIP_2 within 15 sec (they did not measure the accumulation of inositol phosphates in that study). We do not know the cause for this apparent difference in ours and Ochi et al 's (17) observation, but a possible explanation for this difference could be provided by the recent report of Winicov and Gershengorn (19). They compared the inositol trisphosphate formation and cytosolic calicum increases following thyrotropin-releasing hormone and carbamylcholine stimulation in various clones of mouse pituitary tumor cells.





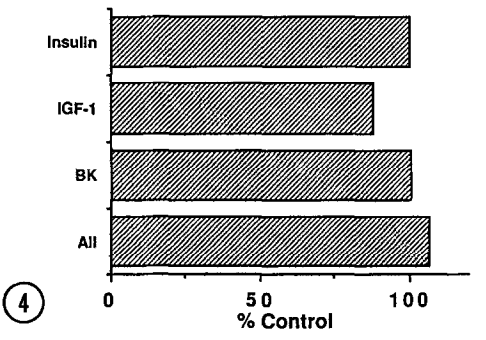


Fig 3. Time-course of AII-stimulated inositol phosphates formation. Inositol labeled glomeruli were divided into two equal portions; to one portion only 10 mM lithium was added (open symbols); the other portion contained 10 mM lithium plus 10 $^{-7}$M AII (closed symbols).

Fig 4. Comparison of angiotensin, bradykinin (BK), insulin and IGF-1 coupling to inositol lipids in glomeruli and tubules. The agonists were employed at a concentration of 10 $^{-7}$M. Concentration of phorbol myristate acetate was 10 $^{-8}$M. Results represnt the mean of duplicate observations.

Though both these agonists stimulate inositol lipid breakdown, inositol trisphosphate accumulation and its accompanying cytosolic calcium release was observed following only thyrotropin-releasing hormone stimulation, but not carbachol. They concluded that a minimal number of receptor-ligand complex was required to see a detectable increases in inositol trisphosphate and the subsequent increases in calcium.

Comparison of the coupling of the phosphoinositide pathway to various receptors in glomeruli (top panel) and tubules (bottom panel) is shown in Fig. 4. In glomeruli, the only positive response besides AII was observed with bradykinin. Bradykinin (10^{-7}M) increased inositol monophosphate formation by 47±10% (3). Bradykinin-stimulated phosphoinositide signal in papillary collecting tubules has been reported previously (20). In endothelial cells (21), bradykinin (1uM) causes a decrease in labeled phosphatidylcholine and an increase in labeled choline through activation of phosphatidylcholine-specific phospholipase C. Both AII- and bradykinin-stimulated inositol phosphate accumulation is inhibited in the presence of phorbol myristate acetate (Fig. 4). Similar inhibition of AII-stimulated response by phorbol ester has been reported in cultured mesangial cells (4).

Insulin receptors have been demonstrated in both glomeruli and tubules of rat kidney. The functional response associated with insulin receptor stimulation, as well as its molecular mechanism of action, are unknown. The lack of insulin-stimulated inositol phosphate accumulation in glomeruli (Fig 4, top panel), is consistent with the finding in other tissues (22) . Insulin has only been shown to cause increases in the level of PI, PIP and PIP_2 (23) as well as PI-glycan (24) in BC_3H1 cells. Recent evidence indicates that at least some of the actions of insulin are mediated through the breakdown of a novel phosphatidylinositol-glycan molecule, resulting in the formation of inositol phosphate glycan (25). IGF-1 (10^{-7}) failed to produce any IP accumulation in glomeruli or tubules. This finding is consistent with the recent observation of Crljen and Banfic (26), who

reported that IGF-1, like insulin, produced a 20% increase in phosphoinositides in rat renal cortical slices, which was not accompanied by inositol phosphate formation.

Our studies clearly demonstrate that AII receptors in glomeruli, but not in tubules, are coupled to phosphoinositide turnover. The above differences are consistant with the presence of different subtypes of AII receptors in glomeruli and tubules (20). The receptors of glomerular mesangiμMare classified as subtype 'A' - which has high affinity for AII and the signal is mediated through phospholipase C-mediated calcium movement. The receptors in tubular epithelial cells are of subtype B, which have lower affinity for AII and the receptor stimulation is accompanied by inhibition of adenylate cyclase (27).

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