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PROSTAGLANDIN BIOSYNTHESIS DOES NOT PARTICIPATE IN ISOPROTERENOL-INDUCED RENIN RELEASE

Supatra Lohsiriwat and Richard L. Malvin

Department of Physiology
University of Michigan
Ann Arbor, Michigan

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Summary

Rat renal slices were incubated in two different media. One was a normal K, physiological saline solution and the other a high K medium. Renin release was measured every 15 min in the presence and absence of 10^{-6}M isoproterenol and also in the presence and absence of aspirin, 0.8 or 1.6 X10^{-5}M. In all experiments renin release was linear during the 75 min of incubation. Isoproterenol increased renin release by approximately 100%. This was the case even in the presence of aspirin which significantly inhibited prostaglandin release (PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$). Nor was there any reduction in the basal secretory rate by aspirin alone. These data are taken to indicate that aspirin in pharmacological doses does not interfere with either in vitro basal release rates of renin, nor the response to B agonists. It is also suggested that B agonists do not exert their effect by stimulating prostaglandin secretion.

Among the many stimuli that may alter the renal secretory rate of renin are adrenoreceptor agonists (1,2) and the rate of production of prostaglandins (3); both stimulate release. Furthermore, it has been reported that the stimulation affected by β agonists is attenuated or prevented by the previous administration of inhibitors of prostaglandin (PG) synthesis: indomethacin or meclofenamate (4-6). Such data have been obtained from conscious rats (4), cats (5) or superfused rat renal cortical slices (6). However, conflicting data have been reported using similar preparations. Pretreatment with meclofenamate or indomethacin has been reported to have no effect on the stimulation of renin secretion by β agonists in the following experimental models: in vivo in rats and dogs (7,8), isolated perfused rabbit kidney (9) and on isolated rat glomeruli (10). Thus it is not clear what relation exists between the two systems.

This study was undertaken in an attempt to obtain more informaton concerning this controversy. We have used rat renal cortical slices as the experimental model and aspirin as the drug of choice for inhibition of PG synthesis. It has fewer side effects than the more potent inhibitors of PG synthesis, meclofenamate and indomethecin. Indomethecin has the additional disadvantage of being a drug which may affect intracellular concentrations of Ca (11). Isoproterenol (ISP) was used as the β agonist to stimulate renin release from the slices. The rates of release for both renin and prostaglandins were measured in all our experiments. In addition, we have used two experimental models. Slices were incubated in solutions containing low (normal) K concentrations, or in high K solutions which reduce the basal secretory rate of renin (12).

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Methods

Male Sprague-Dawley rats, weighing between 200-250 g were used for all experiments. The rats were anesthetized with ether; a dorsal incision was made and bilateral nephrectomy performed. The kidneys were immediately placed in a beaker of ice cold isotonic saline, cut by half transversely, and sliced using a Stadie-Riggs microtome. Each kidney yielded 2-4 slices, approximately 0.4 mm thick.

The slices were preincubated in a flask containing 30 ml of a physiologic saline solution (PSS) at pH 7.4, aerated with 95% O_2 and 5% CO_2 at 37°C for 15 min. Following preincubation the slices were transfered to Erlenmeyer flasks containing 6 ml of PSS (one slice per flask). Incubation lasted 75 minutes. Media samples (100 ul, collected into chilled microtubes) were taken from all flasks at 0, 15, 30, 45, 60 and 75 min, and stored at -20°C for later renin assay. At the end of the incubation period, the slices were quickly removed, blotted and weighed. The medium in each flask was also stored at -20°C for prostaglandin (PG) assays. Since the volume of media needed for PG assay was large compared to that for renin, only the 75 min value was obtained.

The incubation solution was of 2 kinds, normal K^+ (4.7 mM) and high K (35 mM). In mM the normal K solution or PSS was as follows: NaCl 118, KCl 4.7, $NaHCO_3$ 25, $CaCl_2$ 2.5, KH_2PO_4 1.2, MgSO .2 and glucose 10. The high K PSS was the same as above except that additional KCl was added to bring the final K concentration to 35 mM.

The concentration of isoproterenol used to stimulate renin secretion was 10^{-6}M. Prostaglandin synthesis was inhibited by addition of aspirin (acetyl salicylic powder) to make a final concentration of either 0.8 or 1.6 x 10^{-5}M. The concentrations selected were the highest therapeutic level recommended, beyond which toxicity may be expected, 150-300 mg/ml, or 0.8 - 1.6 X 10^{-5}M (13). The stock solution was titrated with NaOH until the aspirin completely dissolved (fluid pH 7.5-7.8). ISP was added at zero time, and aspirin at 15 min, so that we could verify that the tissue was viable during the first 15 min. This method allowed us to obtain curves showing the rate of release. If aspirin had an effect on renin release, it would be manifest by a change in slope.

The frozen incubation media were thawed and centrifuged at 4°C. Five to 30 ul of the media were added to 333 ul of diluted rat renin substrate and the mixture was incubated at 37°C for 10 and 20 min to determine renin activity as previously reported (2). Angiotensin I generated in the mixture was measured by the method of Haber et al. (14), using a New England Nuclear angiotensin I radioimmunoassay kit. Renin released into the medium is expressed as ng angiotensin I generated per mg kidney slice weight per hour (ng AI/mg·hr)

All prostaglandin assays were done by the Diabetic Research and Training Center of the University of Michigan Hospital using a radioimmunoassay. For PGE_2 and PGF_2 the standards and antibodies were obtained from Upjohn Company (Kalamazoo) and tracer from New England Nuclear. For 6-keto-$PGF_1\alpha$ analysis, the [3H] RIA kit from New England Nuclear was used.

Statistics: The 75 min values were compared using Student's t test.

Results

Figure 1 shows the results using the normal K medium. The rate of renin release was linear in all experiments, for all groups. In addition, it is evident that aspirin (0.8×10^{-5}M) had no effect on the basal rate of renin release, nor on the ISP stimulated rate. In the absence and presence of aspirin ISP significantly stimulated renin secretion by 110% and 155% respectively. Although the ISP stimulation was slightly greater in the presence of aspirin that difference was not statistically significant (P=.4).

In contrast to the data for renin those for prostaglandin showed a significant reduction of PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ release on aspirin treatment (Table I). In neither case did ISP alone inhibit release of PG. However, the combination of ISP+aspirin was more effective than aspirin alone in reducing PGE_2 release (P=.03) and $PGF_{2\alpha}$ release (P< .01).

Prostaglandin Release from Slices Incubated in the Low K PSS. Values Are pg/mg tissue at 75 min ± SE

	PGE_2	$PGF_{2\alpha}$	6-keto-$PGF_{1\alpha}$
Control N=18	113±19	19±3	---
Aspirin N=18 (0.8×10^{-5}M)	73±10*	12±1.4*	---
ISP N=12	121±21	14±1	14.3±2.1
Aspirin +ISP N=12 (0.8×10^{-5}M)	46±5**	7.0±.8**	5.4±1.3**
% Inhibition+	77	62	78

* P < .05

** P < .003

+% inhibition was calculated on the assumption that PG release was the same in the first 15 min in the flasks with and without aspirin. Thus, % inhibition = 1-[aspirin - 0 control]/0.8 control.

TABLE II

Prostaglandin Release from Slices Incubated in Low and High K PSS. Values are pg/mg Tissue at 75 min±SE.

	PGE_2	$PGF_{2\alpha}$	6-keto-$PGF_{1\alpha}$
Control low K	149±31	19±1.8	18±3
Control high K	159±30	19±3.5	19±2.6
High K+ISP	112±16	15±1.6	18±3
High K+ISP, aspirin (1.6×10^{-5}M)	50±10**	10±1.6*	9±3**
Mean % inhibition	69	48	62

* P < .03

** P < .01

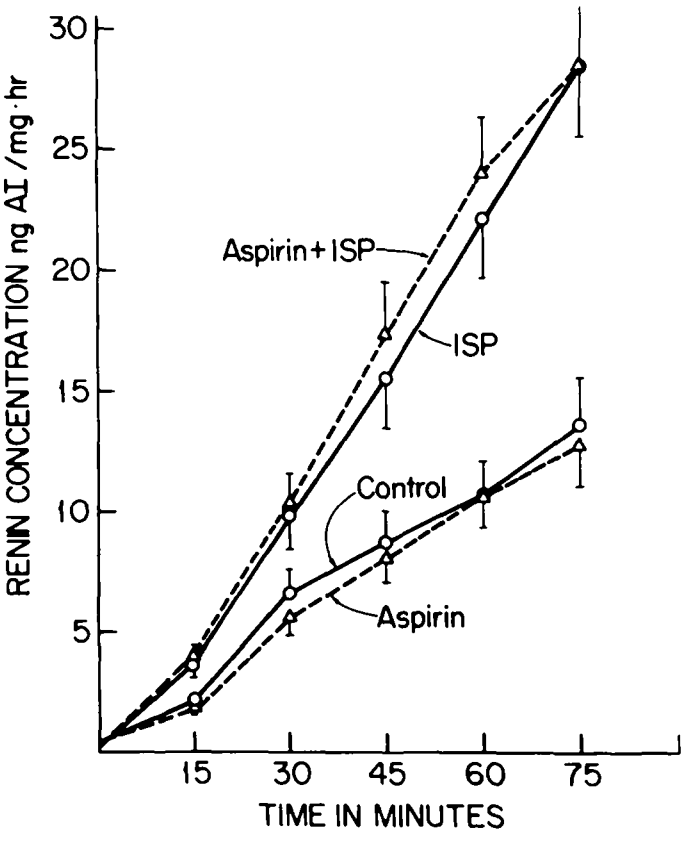

FIG. 1
Renin release as a function of time in normal K PSS. Isoproterenol stimulated release was significantly greater than its appropriate control in both cases. For control vs ISP, $P < .001$. For aspirin control vs aspirin + ISP, $P < .001$. N=18 for control and aspirin groups. N=12 for ISP and ISP+aspirin groups. The concentration of aspirin was 0.8×10^{-5}M.

Data for the experiments using high K PSS are shown in Figure 2. For convenience the rate of another series incubated in normal K PSS is also shown. High K reduced the basal secretory rate to 41% of that in normal K PSS. No change in slope is evident between slices incubated in high K media, ISP with or without aspirin. At 75 min ISP stimulated the renin secretory rate 103% and 75% in the absence and presence of 1.6×10^{-5}M aspirin. The difference was not significant ($P < .25$). Furthermore, no change in slope is evident after aspirin. However, aspirin reduced all three prostaglandin release rates significantly. The concentration of K in the medium did not effect PG release, nor did ISP alone ($P < .2$ for both PGE_2 and $PGF_2\alpha$ and .5 for 6-keto $PGF_1\alpha$). Table II shows the prostaglandin release rates for those experiments.

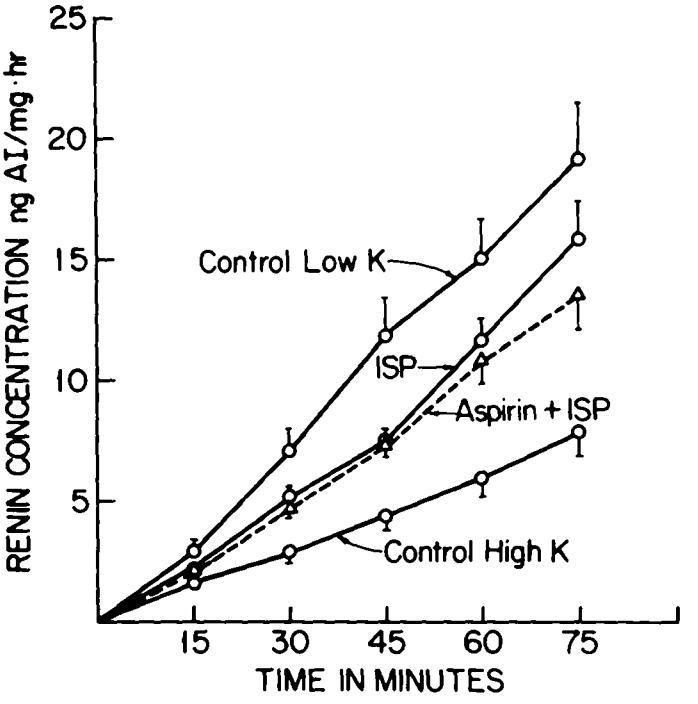

FIG. 2

Renin release in low and high K PSS. ISP significantly increased renin release from slices in the high K PSS both in the absence and presence of aspirin. $P < .001$ in both cases. However, no significant difference exists between the release rates for ISP alone and ISP plus aspirin (1.6×10^{-5} M). For all cases N=12.

Discussion

It has been shown that renal sympathetic nerves and prostaglandins are important modulators of renin release (15). It has also been shown that increased concentrations of prostaglandins stimulate renin secretion both in vivo (16) and in vitro (17). Although a few investigators have published data indicating that inhibition of PG synthesis with meclofenamate or indomethacin attenuates the stimulatory effect of ISP (4-6), others have shown no effect of such PG inhibition (7-10). No reason is apparent for these discrepant results. However, it is possible that in vivo or whole kidney experiments, were complicated by hemodynamic changes since four important variables, GFR, RPF, sodium excretion and blood pressure were not measured in most of the experiments. In addition the cyclooxgengase inhibitors used were meclofenamate or indomethecin, both of which exhibit more nonspecific effects than aspirin (18). Our work used aspirin in 2 concentrations, both of which were effective in inhibiting PG synthesis. Furthermore, in vitro experiments were done using two different media, a low or normal and a high K PSS. High K media is presumed to inhibit renin release by causing an increase in the intracellular concentration of Ca (19). In both situations the results were the same. Aspirin significantly inhibited the release, and presumably the synthesis of PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$.

However, in spite of the significant inhibition of PG synthesis, no effect on the basal secretory rate of renin release, or on the ISP stimulation of renin release was noted. Since these experiments are uncomplicated by alterations in baroreceptor stimulation, we believe no evidence is apparent to conclude that β agonists affect renin secretion by first causing an increased release of PGs. It should also be noted that PG synthesis did not vary with basal renin secretory rates. Renin release varied by a factor of two in high vs. low K media, but PG release was virtually identical. Although in these experiments PG release appears to have no significant effect on renin release, there are other situations in which they seem to be related (3-6). We conclude that whatever the mechanism of in vitro ISP stimulation of renin release is, it is independent of prostaglandin synthesis.

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