The Mechanism of the Control of Renin Release by Beta-Adrenergic Receptors

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SUMMARY

Recent reports suggest that prostaglandins play an important role in the beta-adrenergic receptor mechanism of renin release. However, the site of the action of prostaglandins has not yet been clarified. Superfusion of rabbit renal cortical slices was used to evaluate the beta-adrenergic receptor mechanism of renin release. Renin release was stimulated by isoproterenol, prostaglandin E₂, and dibutyryl cyclic AMP. Renin release stimulated by isoproterenol was inhibited by propranolol, whereas renin release stimulated by prostaglandin E₂ was not inhibited by propranolol. Isoproterenol stimulated prostaglandin E₂ release as well as renin release, and indomethacin inhibited these effects of isoproterenol. Propranolol inhibited prostaglandin E₂ release stimulated by isoproterenol. On the other hand, indomethacin did not affect renin release stimulated by prostaglandin E₂. Dibutyryl cyclic AMP did not stimulate prostaglandin E₂ release. Indomethacin did not affect renin release stimulated by dibutyryl cyclic AMP, however, it suppressed prostaglandin E₂ release during the superfusion with dibutyryl cyclic AMP. Finally, isoproterenol and prostaglandin E₂ stimulated cyclic AMP release. These data suggest that prostaglandins play an important role in the beta-adrenergic receptor mechanism of renin release and the site of the action of prostaglandins is between the beta-adrenergic receptor and cyclic AMP.

Additional Indexing Words:
Renin Beta-adrenergic receptor Prostaglandins Cyclic AMP Indomethacin

RENIN release is controlled by many factors such as the beta-adrenergic receptor mechanism, baroreceptor mechanism, macula densa mechanism and prostaglandins. It is well established that isoproterenol, a sympa-
thetic beta-adrenergic receptor agonist, stimulates renin release via the adeny-
late cyclase-cyclic AMP system. Recent reports suggest that prostag-
landins play an important role in the control of renin release. It has
also been reported that various kinds of prostaglandins stimulate renin release
in vitro and in vivo. Our previous study in rats indicated the possibility that prostaglandins play an important role in the beta-adrenergic
receptor mechanism of renin release and that the site of the action of pros-
taglandins is between the beta-adrenergic receptor and cyclic AMP.

However, it has not been studied whether or not propranolol inhibits
prostaglandin production stimulated by isoproterenol and whether or not pros-
taglandins stimulate cyclic AMP production in the kidney. The present study
was carried out to obtain further information about the precise mechanism
of control of renin release by the beta-adrenergic receptor, and also to study
the role of prostaglandins in this mechanism.

**Materials and Methods**

White male rabbits weighing 2.0–3.0 kg were used for the experiments. Rabbits were maintained on a regular diet and were anesthetized with sodium pentobarbital (25 mg/kg i.v.). The kidneys were quickly removed, decapsu-
lated, and placed in a Stadie-Riggs microtome. One renal cortical slice, 0.3–0.5 mm in thickness, was obtained from the ventral and another from
the dorsal surface of each kidney. Eighty mg of renal cortical slice were
placed in the middle of two o-ring joints that contained a small plastic filter
in one side to prevent displacement of the tissue after the superfusion was
started. The two o-ring joints were held together by a clamp and the two
ends of it were connected with polyethylene tubing (PE280) with an interior
diameter of 2.15 mm. The tissue holder was perfused with Krebs-Ringer
bicarbonate glucose buffer (KRBG), pH 7.4, containing Na+, 144 mM; Ca++,
2.7 mM; Cl-, HCO-, 25 mM; Mg++, 1.2 mM; glucose, 11 mM, which was
continuously bubbled with a mixture of 95% O₂ and 5% CO₂. The polyethy-
lene tubing passed through a peristaltic pump. The tubing was connected
to a warming coil and a debubbler before reaching the tissue. The efferent
end of the tubing was connected to a fraction collector. The dead space of
the whole system was about 10 ml and the flow rate was 1 ml/min. The
initial 75 min of superfusion with KRBG were discarded. After a 60 min
superperfusion to obtain a stable baseline, a control sample was collected over
a 20-min period. The agents were dissolved in 30 ml of KRBG and super-
fused over 30 min. The experimental sample was collected during the last
20 min after application of the stimulus. The superfusate was changed to the
control KRBG and the next control sample was obtained during the last 20 min of the 30 min control superfusate. The above schedule was repeated 2 to 3 times. Experiments were performed with isoproterenol (8×10^{-7} M), propranolol (2×10^{-5} M), PGE_{2} (10^{-6} M), dibutryl cyclic AMP (10^{-3} M), and indomethacin (10^{-4} M). One mg/ml of bovine serum albumin was added to each superfusion sample just after collection to prevent the destruction of renin.

Renin concentration in superfusion samples was measured by radioimmunoassay using a modification of Haber's method.\textsuperscript{22} Fifty µl of superfusion samples were incubated with 75 µl of nephrectomized sheep plasma, 90 µl of Tris-acetate lysozyme buffer, pH 7.4, 25 µl of 4% EDTA, 5 µl of dimercaprol, and 5 µl of 8-hydroxyquinoline. Incubation was for 3 h at 37°C, after which samples were transferred to an ice bath and 50 µl aliquots were taken for radioimmunoassay of generated angiotensin I (CEA-IRASORIN Co., Renin Kit). Prostaglandin E\textsubscript{2} was measured by radioimmunoassay as previously described by us.\textsuperscript{17} Cyclic AMP was measured by radioimmunoassay using a cyclic AMP kit (Yamasa Co.). Data were analyzed by the paired Student's t-test and significance was accepted at p<0.05.

\textbf{RESULTS}

Fig. 1 shows the effects of isoproterenol (8×10^{-7} M), PGE_{2} (10^{-5} M),

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Effects of isoproterenol (Isp), PGE_{2}, and dibutryl cyclic AMP (DB-CAMP) on renin release (RR) from renal cortical slices. Isp, PGE_{2}, and DB-CAMP stimulated RR significantly. B=baseline level.}
\end{figure}
Fig. 2. Effects of propranolol (Prop) on renin release (RR) stimulated by isoproterenol (Isp) and PGE₂. Prop inhibited RR stimulated by Isp, however, it did not affect RR release stimulated by PGE₂. B=baseline level.

Isoproterenol stimulated renin release from 17.6±3.3 to 35.5±5.1 ng/ml/hr (p<0.01). PGE₂ stimulated renin release from 17.4±2.5 to 35.6±2.6 ng/ml/hr (p<0.01). Dibutyryl cyclic AMP also stimulated renin release from 15.2±2.8 to 38.1±2.1 ng/ml/hr (p<0.01).

Fig. 2 shows the effects of propranolol on renin release stimulated by isoproterenol and by PGE₂. Propranolol inhibited renin release stimulated by isoproterenol, however, it did not inhibit renin release stimulated by PGE₂.

Fig. 3 shows the effects of isoproterenol on renin release and PGE₂ release. Isoproterenol stimulated renin release and PGE₂ release (p<0.005). Indomethacin inhibited renin release and PGE₂ release stimulated by isoproterenol. Propranolol also inhibited renin release stimulated by isoproterenol. Propranolol inhibited prostaglandin E₂ release stimulated by isoproterenol.
Fig. 3. Effects of isoproterenol (Isp) on renin release (RR) and PGE₂ release. Isp stimulated RR release and PGE₂ release. Indomethacin (Indo) inhibited RR stimulated by Isp. Indo also inhibited PGE₂ release stimulated by Isp. Propranolol (Prop) inhibited RR stimulated by Isp. Prop also inhibited PGE₂ release stimulated by Isp. B = baseline level.

Fig. 4. Effects of indomethacin (Indo) on renin release (RR) stimulated by PGE₂. Indo did not affect RR stimulated by PGE₂. B = baseline level.
Fig. 5. Effects of dibutyryl cyclic AMP (DB-CAMP) on renin release
and PGE$_2$ release. DB-CAMP stimulated RR release, however, it did not
stimulate PGE$_2$ release. Indomethacin (Indo) did not affect RR stimulated
by DB-CAMP, however, it suppressed PGE$_2$ release during DB-CAMP superfusion. B=baseline.

Fig. 4 shows the effects of indomethacin on renin release stimulated by
prostaglandin E$_2$. Indomethacin did not affect renin release stimulated by
prostaglandin E$_2$.

Fig. 5 shows the effects of dibutyryl cyclic AMP on renin release and
PGE$_2$ release. Dibutyryl cyclic AMP stimulated renin release significantly
(p<0.01) but not PGE$_2$ release. Indomethacin did not affect renin release
stimulated by dibutyryl cyclic AMP, however, it suppressed PGE$_2$ release
during dibutyryl cyclic AMP superfusion.

Fig. 6 shows the effects of isoproterenol and PGE$_2$ on cyclic AMP release
from renal cortical slices. Isoproterenol (8×10$^{-7}$ M) stimulated cyclic AMP
release from 0.31±0.04 to 0.60±0.05 pmol/ml (p<0.05). PGE$_2$ (10$^{-5}$ M)
also stimulated cyclic AMP release from 0.32±0.05 to 0.59±0.04 pmol/ml
(p<0.05).
Fig. 6. Effects of isoproterenol (Isp) and PGE2 on cyclic AMP (C-AMP) release. Isp and PGE2 stimulated C-AMP. B=baseline level.

**DISCUSSION**

It has been established that beta-adrenergic agents play an important role in the control of renin release. Isoproterenol was reported to stimulate renin release via cyclic AMP production. The role of prostaglandins in the beta-adrenergic receptor mechanism of renin release is controversial and needs further study. Seymour et al and Berl et al reported that indomethacin did not inhibit renin release stimulated by isoproterenol in dogs in vivo. These results in the dog are in agreement with studies in man by Frolich et al. However, our previous report suggested that indomethacin inhibited renin release stimulated by isoproterenol in rats in vitro. The present study in rabbits in vitro clearly shows that prostaglandins play an important role in the beta-adrenergic receptor mechanism of renin release since indomethacin inhibited renin release stimulated by isoproterenol. It seems to be difficult to explain the difference between their data and ours. In our in vitro experiments, but not in their in vivo experiments, hemodynamic effects of isoproterenol and indomethacin were avoided. Furthermore, Campbell et al and Feuerstein et al reported that indomethacin inhibited renin release stimulated by isoproterenol in in vivo studies of rats and cats, respectively, findings confirmed by our previous study in rats in vitro. Therefore, the above differences may be species specific. The present study indicates...
that prostaglandins are related to the beta-adrenergic receptor mechanism of renin release. The present study clearly shows that PGE₂ stimulates cyclic AMP release as well as renin release. We have previously shown that theophyllin (4×10⁻³ M) had no effect on renin release, but when added to subthreshold doses of prostaglandin E₂ (10⁻⁹ M), it stimulated renin release significantly. These data suggest that prostaglandins stimulate renin release via cyclic AMP production. Our present data demonstrating that isoproterenol stimulates prostaglandin E₂ release as well as renin release and that these actions of prostaglandin E₂ are inhibited by propranolol indicate that isoproterenol stimulates renin release via prostaglandin production. The data showing that indomethacin inhibited renin release stimulated by isoproterenol suggest this conclusion. Using superfused rat glomeruli, however, Beierwaltes et al. showed that isoproterenol stimulated renin release without increasing prostaglandin E₂ release. The above discrepancy may be explained by the fact that the renal tubular cells are much richer in prostaglandin E₂ than are the glomerular cells. Our present study suggests that isoproterenol stimulates renin release via prostaglandin production. These data agree with the previous reports by Campbell et al. that renin release stimulated by isoproterenol was blocked by indomethacin. However, they reported that indomethacin also inhibited renin release stimulated by dibutyryl cyclic AMP and their conclusion was that prostaglandins are involved in the beta-adrenergic receptor mechanism of renin release and that the site of action of prostaglandins is distal to cyclic AMP. In contrast to their data, the present study clearly shows that indomethacin did not inhibit renin release stimulated by dibutyryl cyclic AMP in vitro. Our data suggest that prostaglandins stimulate renin release via cyclic AMP production. Prostaglandins were reported to stimulate the production of cyclic AMP in several tissues and the present study suggests that they stimulate renin release via cyclic AMP production.

These data suggest that prostaglandins play an important role in the beta-adrenergic receptor mechanism of renin release and that the site of action of prostaglandins is between the beta-adrenergic receptor and cyclic AMP.

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