Effects of Saline Infusion on Prostaglandin-like Materials in Renal Venous Blood and Medulla of Canine Kidney

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SUMMARY

An activity of prostaglandin (PG)-like substances was measured in venous blood and renal medullary tissue of dogs. The PG-like substances were extracted by silica gel column chromatography and their activity was measured by rat vasodepressor bioassay. The activity of PGE-like substance both in renal venous blood and medulla was greater in saline infused dogs than in control, non-infused dogs. The possibility was discussed that the increased activity of PGE in kidney may be involved in the mechanisms of natriuresis in saline-loaded animals.

Additional Indexing Words:
Blood volume expansion Natriuretic factors Natriuresis

It has been demonstrated that prostaglandin E and A increase renal blood flow, urine flow and sodium excretion. Prostaglandin E (PGE) has also been shown to inhibit the effect of vasopressin in vitro and possibly in vivo. Natural occurrence of prostaglandins in renal tissue together with their effects on kidney functions suggested that prostaglandins may play a physiological role in the regulation of sodium and water excretion.

The present experiments were designed to examine the effects of saline infusion on prostaglandins concentration in the renal venous blood and renal medullary tissue.

METHOD

Collection of blood and renal tissue:
Female mongrel dogs weighing 7–12 Kg, fasted and thirsted overnight prior to the experiments, were anesthetized with sodium pentobarbital (25 mg/Kg, iv). The left kidney was exposed retroperitoneally by the flank incision and the left ureter was catheterized. After the infusion of 0.9% NaCl (150 ml/Kg) into the cubital vein for 120 or 150 min, the needle connected with plastic tube was inserted into the left renal vein and about 200 ml of blood was withdrawn into a syringe containing

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20,000 U.S.P. units of heparin and 2 Gm of L-ascorbic acid. The blood was rapidly introduced into a plastic beaker containing 240 ml of cold chloroform-methanol (1:4 v/v). Both kidneys were immediately removed, medullary tissues were excised, weighed and put into 4–10 volumes of cold chloroform-methanol (2:1, v/v). In control experiments, samples were taken without saline loading.

**Extraction and purification:**

Extraction of PGE and PGA from the blood was carried out by the modification of the method of Edwards et al. Blood in chloroform and methanol was homogenized, supernate was decanted and the precipitate was washed with 400 ml of chloroform. The supernate and the chloroform were combined, adjusted to pH 3.0 and the aqueous phase was removed. After washing with distilled water, the chloroform extract was evaporated to dryness at 40°C. Extraction from tissue was performed similarly, as shown in Fig. 1. The extracted materials both from tissue and blood were purified using the method of Edwards et al. In short, dried crude extract was dissolved in a small amount of chloroform and put on the silica gel column. After washing with 50 ml of chloroform, PGA fraction was eluted with 50 ml of methanol: chloroform (3:97, v/v). Then, PGE fraction was eluted with 50 ml of methanol: chloroform (20:80, v/v). In order to obtain enough prostaglandin activity for bioassay, each fraction of eluates had been stored at -20°C, until the experiments were performed on 5 dogs and the extraction was done in total on 900–950 ml of blood and 70–80 Gm of tissue. Then, the eluted PGE and PGA fractions were pooled, again evaporated to dryness at 40°C and dissolved in 2.0 ml petrolitum ether. To it 0.5 ml of 0.2 M phosphate buffer (pH 8.0) was added. The aqueous phase was used for bioassay and silica gel thin-layer chromatography. The use of 20% ethanol to dissolve dried extract was avoided because ethanol itself caused blood pressure depression in the assay animal. The solvent systems of the thin-layer chromatography were of the same composition as those of Edwards et al., except the silver nitrate impregnation was not performed.
Fig. 2. Typical dose response curve in the assay animal (nephrectomized rat). The magnitude of initial depression in carotid artery blood pressure was plotted against the dose of PGE₁ injected into the femoral vein.

Table I. The Activity of PGE- and PGA-like Substances (PGE-LS, PGA-LS) of the Renal Venous Blood and Renal Medulla in Control and Saline-loaded Dogs (Assayed as PGE₁ Equivalents)

<table>
<thead>
<tr>
<th>No. of Measurements</th>
<th>Renal Venous Blood</th>
<th>Renal Medullary Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PGE-LS (ng/ml)</td>
<td>PGA-LS (ng/ml)</td>
</tr>
<tr>
<td>Control dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>&lt;0.20*</td>
<td>&lt;0.20*</td>
</tr>
<tr>
<td>2</td>
<td>0.25</td>
<td>&lt;0.20*</td>
</tr>
<tr>
<td>3</td>
<td>0.20</td>
<td>&lt;0.20*</td>
</tr>
<tr>
<td>Mean</td>
<td>0.22</td>
<td>&lt;0.20*</td>
</tr>
<tr>
<td>Saline-loaded dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.44</td>
<td>1.29</td>
</tr>
<tr>
<td>2</td>
<td>0.84</td>
<td>&lt;0.20*</td>
</tr>
<tr>
<td>3</td>
<td>0.56</td>
<td>0.51</td>
</tr>
<tr>
<td>Mean</td>
<td>0.95</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Significance of difference between means (t-test)  

|                              | P < 0.05 | NS     | P < 0.05 | NS     |

In each measurement pooled extract from 900–950 ml of blood or 70–80 Gm of tissue was used for bioassay.

*, **: PG activity was not detected. The values under the stars were determined by the bioassay sensitivity and used for the calculation of mean and t-test.
Bioassay:
Rats nephrectomized 18-22 hours prior to the assay, were anesthetized with sodium pentobarbital. Trachea was cannulated for free airway. Right carotid artery was cannulated for continuous recording of blood pressure. Femoral vein was cannulated for the injection of assay materials. A typical dose response curve was shown in Fig. 2. The minimum sensitivity was 0.2μg of PGE₁ or less, according to the assay animals. Activities of PGE- and PGA-like substances in the purified extracts were measured by bracket assay.

Results
The data are shown in Table I. The activity of PGE-like substance both in the renal venous blood and medullary tissue was significantly higher in the saline-infused dogs than in control dogs (P<0.05, t-test). No significant difference was detected in the activity of PGA-like substance between the control dogs and saline-infused dogs. The recovery of PGE₁ added to the blood was 26±6%. The data were not corrected for the recovery. Although PGE and sometimes PGA-like substances were detected in the extracts by silica gel thin-layer chromatography, further identification of these substances could not be carried out. PGE activity was not measured. Table II shows an average change in urine flow, urine osmolality and sodium excretion in the left kidney before and at the end of isotonic saline infusion.

Table II. Effect of Isotonic Saline Infusion on Urine Flow (V), Urine Osmolality (Uosm), Plasma Osmolality (Posm), Sodium Excretion (Uₙₐᵥ) and Potassium Excretion (Uₖᵥ)

<table>
<thead>
<tr>
<th></th>
<th>Before Infusion</th>
<th>After Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>V (ml/min)</td>
<td>0.23±0.10</td>
<td>5.15±0.67</td>
</tr>
<tr>
<td>Uosm (mOsm/Kg H₂O)</td>
<td>963±129</td>
<td>294±12</td>
</tr>
<tr>
<td>Uₙₐᵥ (μEq/min)</td>
<td>48.8±20.1</td>
<td>911.2±117.9</td>
</tr>
<tr>
<td>Uₖᵥ (μEq/min)</td>
<td>7.3±0.4</td>
<td>31.7±3.4</td>
</tr>
<tr>
<td>Posm (mOsm/Kg H₂O)</td>
<td>300.3±1.3</td>
<td>294.3±4.1</td>
</tr>
</tbody>
</table>

Mean±SE. Urine was collected from the left ureter.

Discussion
It has been demonstrated that prostaglandins are present in most tissues and their release from tissues can be increased by neural and hormonal stimulation⁹) or by mechanical stimuli like stretch of tissues.⁹) In the dog, renal nerve stimulation¹⁰) or infusion of angiotensin¹¹) or noradrenaline¹²) has been shown to enhance the release of prostaglandins into the renal venous outflow.
Furthermore, the renal blood flow which had been reduced by the continued infusion of such a vasoconstrictive substance was shown to return towards its control level, coincident with the release of prostaglandins.10) Recently, it was found that bradykinin, a kind of vasodilator, increased the concentration of PGE-like substance in the renal venous blood, when infused into the renal artery of the dog.13) The results of the present experiments showed a higher PGE-like activity in the medulla and renal venous outflow of saline-loaded dogs, suggesting that possible production and release of PGE in the kidney13 may be enhanced by saline infusion. It was also found that the level of PGE in the present experiment roughly corresponded to the data of previous reports,6),10)-13) although methods of extraction and assay were different.

In saline diuresis, there occurs an increase in renal blood flow (RBF)14 and possibly a redistribution of intrarenal blood flow.15) It has already been pointed out that the large increase in RBF would require a striking decrease in the resistance to blood flow through the kidney, since arterial blood pressure is not increased during saline infusion.12) PGE and PGA infused into the renal artery of the dog have been reported to cause an increase in RBF, predominantly in cortical flow,16),17) accompanied by an increase in sodium excretion. The threshold dose of PGE1 for such responses was reported to be as low as 20 ng/min, when infused into the renal artery of the dog.18) On the other hand, it has been demonstrated that PGE1, contrary to the so-called "natriuretic plasma" in some experiments,19) stimulates sodium transport across the toad bladder.20) Therefore, supposing that the vasodepressor materials in the present experiments are prostaglandins, increased release of PGE in the saline-loaded dog might be responsible, in part, for the increased sodium excretion, possibly through their effects on renal hemodynamics. The effects of formed or released PGE, if any, seem to be "local" or intra-renal, since PGE in blood was found to be almost completely inactivated by passing through the pulmonary or portal circulation.21) Of course, many other factors are and should be involved in the mechanisms of saline diuresis, since, for example, an increase in sodium excretion caused by infusion of prostaglandins alone is generally much smaller1),3),4),18) than that caused by saline infusion (Table II). It is also necessary to consider another possibility that prostaglandin release may not be a cause of natriuresis but only a result of biochemical changes in the kidney under saline diuresis.

In spite of the possibility that prostaglandins may mediate the natriuresis following volume expansion,22) so far as we know, the effect of saline infusion on prostaglandin activity in the renal venous blood and medulla has not been reported yet. In the present experiments, its effect was found to be significant (Table I), although a larger number of experiments would be desirable for
the definitive conclusions. For this, more sensitive and specific assays are preferable. Radioimmunoassay of prostaglandins recently being developed may be helpful.

ACKNOWLEDGEMENT

Samples of standard PGE\textsubscript{1}, PGE\textsubscript{2}, PGA\textsubscript{1}, and PGA\textsubscript{2} were supplied by Ono Pharmaceutical Company.

REFERENCES

8. Ramwell PW, Shaw JE: Recent Prog Hor Res 26: 139, 1970