EFFECTS OF PROBENECID, SODIUM SALICYLATE, 2,4-DINITROPHENOL AND PYRAZINAMIDE ON RENAL SECRETION OF URIC ACID IN CHICKENS

BOHDAN R. NECHAY AND LARYSSA NECHAY

Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Florida

Received for publication March 12, 1959

According to classical concepts, uric acid is presumably filtered at the renal glomeruli and reabsorbed but not secreted by the renal tubules in man (Berliner et al., 1950). Recent work (Yü and Gutman, 1959) suggests that uric acid may, however, be both secreted and reabsorbed by the tubules. In this species probenecid and salicylates may either increase renal excretion of uric acid, and the ratio of uric acid clearance to glomerular filtration rate, or decrease these values depending on the dose given (Yü and Gutman, 1955).

In rabbits given exogenous loads of uric acid, the clearance of uric acid is stated to rise above that of creatinine (Poulsen and Praetorius, 1954). In this situation both probenecid and salicylates uric acid depress the clearance ratio (Poulsen, creatinine 1955).

In Dalmatian dogs uric acid appears to be secreted by the renal tubules and probenecid uric acid depresses the clearance ratio (Wolfson et al., 1950; Beyer, 1954).

There is conclusive proof and acceptance by workers in renal physiology that uric acid is excreted mainly by renal tubular secretion in the chicken (Mayrs, 1924; Smith, 1955). For this reason we have used chickens for studies of the effects of certain drugs on the secretion of uric acid.

Methods. Unanesthetized White Leghorn laying chickens, maintained on a commercial laying mash (Purina), were used in this study. The individual birds were allowed at least 2 weeks between experiments.

The birds were tied, heads up, by wings and legs to a small animal surgical table tilted to about 45°. Water (30 to 40 ml/kg/hr) was infused directly into the crop by a plastic feeding tube. Occasionally the rate of infusion had to be diminished to prevent overdistention of the crop and vomiting. After establishment of a sufficient urine flow for the visible masses of undissolved urates to disappear, the urine samples were collected into graduated cylinders by means of a glass tube. Usually one stitch was necessary to keep the funnel-like projection of the collecting tube in the cloaca. The large intestine was blocked with a cotton ball. The para-aminobipurrate (PAH) and inulin were infused and drugs injected through a polyethylene catheter in a wing vein. The other wing vein was cannulated for withdrawal of blood samples. In every experiment there were two control urine collection periods of 15 minutes; all postdrug periods were 30 minutes. At the mid-point of each period, blood samples were collected in syringes which had been rinsed with sodium polyanhydro-mannuronic acid sulfate (100 mg/ml).

Each 15-minute urine sample was immediately made up to 50 ml and each 30-minute sample to 100 ml with distilled water. From this, 1 ml was withdrawn for inulin determinations and the rest of the sample diluted to 100 ml or 200 ml, respectively, with 0.1 N sodium hydroxide for analysis of uric acid and PAH. No correction has been made for withdrawal of urine for inulin determinations.

Plasma and urinary uric acid concentrations were determined on the day of collection by an enzymatic method employing ultraviolet spectrophotometry (Praetorius, 1949; Praetorius and Poulsen, 1953). Only those data for which duplicates checked within 5% were accepted as valid. Inulin was used as a measure of glomerular filtration rate (GFR). It was determined by the diphenylamine method combined with alkali treatment (Little, 1949). Concentrations of PAH were measured by the method of Bratton and Marshall (1939).

Concentrations of probenecid such as used in these experiments did not interfere with any of our analytical methods.
TABLE 1

Representative effects of a single dose of probenecid on uric acid clearance in chickens

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow (ml/min)</th>
<th>Uric acid (mg/min)</th>
<th>Plasma Concentration</th>
<th>Uric acid (mg%)</th>
<th>PAH (mg%)</th>
<th>Plasma Concentration</th>
<th>Uric acid (ml/min)</th>
<th>PAH (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-120</td>
<td>IntraCrop infusion: water 2 ml/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-45)</td>
<td>0.7</td>
<td>1.60</td>
<td>3.3</td>
<td>39</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-30)</td>
<td>0.7</td>
<td>1.20</td>
<td>2.8</td>
<td>32</td>
<td>2.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-15</td>
<td>+5 Probenecid: 100 mg/kg, i.v. (5% solution)</td>
<td>1.2</td>
<td>1.8</td>
<td>7.2</td>
<td>5.1</td>
<td></td>
<td>12.8</td>
<td>16.4</td>
</tr>
<tr>
<td>0-30</td>
<td>1.6</td>
<td>0.80</td>
<td>5.1</td>
<td>27</td>
<td>8.4</td>
<td></td>
<td>16.7</td>
<td>16</td>
</tr>
<tr>
<td>30-60</td>
<td>1.4</td>
<td>0.85</td>
<td>6.0</td>
<td>27</td>
<td>15.6</td>
<td></td>
<td>14.8</td>
<td>9</td>
</tr>
<tr>
<td>60-90</td>
<td>1.3</td>
<td>0.80</td>
<td>13.4</td>
<td>35</td>
<td>24.0</td>
<td></td>
<td>6.4</td>
<td>5</td>
</tr>
<tr>
<td>90-120</td>
<td>1.5</td>
<td>1.00</td>
<td>14.0</td>
<td>29</td>
<td>32.0</td>
<td></td>
<td>7.0</td>
<td>5</td>
</tr>
<tr>
<td>120-150</td>
<td>1.3</td>
<td>1.10</td>
<td>13.6</td>
<td>33</td>
<td>36.0</td>
<td></td>
<td>8.0</td>
<td>6</td>
</tr>
</tbody>
</table>

Experiment 28, chicken 3, weight 3.0 kg

-95 IntraCrop infusion: water 2 ml/min | 1.v. infusion: PAH 8 g/l, inulin 6 g/l, (0.5 ml/min) | 1.7 | 1.92 | 7.6 | 38 | 3.0 | 25 | 6.7 | 50 |

-30 Probenecid: 120 mg/kg, i.v. (5% solution) | 1.2 | 1.24 | 15.3 | 35 | 17.2 | 8 | 4.8 | 6 |

-30-60 | 0.6 | 1.41 | 19.1 | 37 | 26.4 | 7 | 5.3 | 5 |

RESULTS. Control observations. Two types of control experiments were performed. First, the birds were subjected to the procedures described above, and were found to maintain constant clearances of inulin, PAH and uric acid for a period of 4 to 5 hours. Second, single intravenous doses of probenecid 200 mg/kg, and sodium salicylate 300 mg/kg, well in excess of doses used in this study, did not produce any changes in appearance, behavior or appetite of birds as observed for 24 hours, except that urine voided contained smaller than usual amounts of visible precipitates of urates. The birds also tolerated well intravenous doses of 10 mg/kg 2,4-dinitrophenol (DNP) and 100 mg/kg of pyrazinamide.

Probenecid. The effects of probenecid are shown in table 1. These are representative of five similar experiments. At doses of 100 to 120 mg/kg the clearances of uric acid fell sharply, approaching the values for inulin clearance in 30 to 90 minutes. PAH secretion was inhibited almost exactly parallel to that of uric acid. As shown in figure 1,
### TABLE 2

<table>
<thead>
<tr>
<th>Time</th>
<th>Experiment 26, chicken 10, weight 1.8 kg</th>
<th>Experiment 34, chicken 12, weight 1.8 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>-85</td>
<td>Infracrop infusion: water 1.2 ml/min</td>
<td>Sodium salicylate: 200 mg/kg, i.v. (5% solution)</td>
</tr>
<tr>
<td>(-30)-(15)</td>
<td>I.v. infusion: PAH 8 g/l, inulin 6 g/l, (0.5 ml/min)</td>
<td>0-30</td>
</tr>
<tr>
<td>(-15)-(0)</td>
<td>1.3</td>
<td>1.12</td>
</tr>
<tr>
<td>+3</td>
<td>1.6</td>
<td>1.13</td>
</tr>
<tr>
<td>0-30</td>
<td>1.7</td>
<td>1.15</td>
</tr>
<tr>
<td>30-60</td>
<td>1.7</td>
<td>1.20</td>
</tr>
<tr>
<td>60-90</td>
<td>1.7</td>
<td>1.24</td>
</tr>
<tr>
<td>90-120</td>
<td>2.6</td>
<td>1.34</td>
</tr>
<tr>
<td>120-150</td>
<td>1.0</td>
<td>0.77</td>
</tr>
<tr>
<td>150-180</td>
<td>1.0</td>
<td>0.72</td>
</tr>
<tr>
<td>+1</td>
<td>Sodium salicylate: 200 mg/kg, i.v. (5% solution)</td>
<td>Sodium salicylate: 200 mg/kg, i.v. (5% solution)</td>
</tr>
<tr>
<td>0-30</td>
<td>1.9</td>
<td>1.01</td>
</tr>
<tr>
<td>30-60</td>
<td>1.0</td>
<td>0.77</td>
</tr>
<tr>
<td>60-90</td>
<td>0.8</td>
<td>0.96</td>
</tr>
<tr>
<td>90-120</td>
<td>1.0</td>
<td>1.26</td>
</tr>
<tr>
<td>120-150</td>
<td>0.9</td>
<td>1.28</td>
</tr>
<tr>
<td>150-180</td>
<td>0.9</td>
<td>1.31</td>
</tr>
</tbody>
</table>

There was a tremendous retention of uric acid in the plasma, reaching the peak of about four times the control value and returning to predrug concentration in 6 hours. The rise in plasma urate is also notable for its rapid build-up. In the experiment of figure 1, plasma uric acid has risen from 4 mg % to 8 mg % in 15 minutes. In experiment 28, we have recorded a rise from the control average of 7.4 mg % to 15.3 mg % in 15 minutes. In experiment 23, the increase was slower but of the same magnitude.

**Sodium salicylate.** Pilot experiments showed that the highest dose of sodium salicylate that could be used without altering renal hemodynamics was 200 mg/kg. Table 2 shows representative experiments of a series of four; it is evident that sodium salicylate depresses uric acid excretion but the effect is not as striking as that of probenecid. The uric acid clearance returns to control values in approximately 3 hours. This correlates well with the duration of elevated uric acid concentrations in plasma (fig. 1). Our technique did not permit maintenance of water diuresis long enough to observe the return of uric acid clearances to predrug values following the administration of probenecid. There was a modest and transitory depression of PAH secretion.

**2,4-Dinitrophenol.** Previous studies have demonstrated that DNP exhibits a depressant action on the secretory renal tubular transport of PAH, phenol red and diodrast in the dog (Mudge and Taggart, 1950). Table 3 is representative of four experiments which extend this observation to the reduction of uric acid secretion in the chicken.
TABLE 3

Representative effects of a single dose of 2,4-dinitrophenol on uric acid clearance in chickens

<table>
<thead>
<tr>
<th>Time</th>
<th>Urine</th>
<th>Plasma Concentration</th>
<th>Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flow</td>
<td>Uric acid</td>
<td>Uric acid</td>
</tr>
<tr>
<td></td>
<td>ml/min</td>
<td>mg/min</td>
<td>mg %</td>
</tr>
</tbody>
</table>

Experiment 35, chicken 13, weight 2.0 kg

-90 Intracrop infusion: water 1.3 ml/min
  I.v. infusion: PAH 8 g/l, inulin 6 g/l, (0.5 ml/min)
  (-30)-(-15) 1.3 | 1.13 | 2.4 | 33 | 3.6 | 47 | 4.2 | 64
  (-15)-(0) 1.4 | 1.12 | 2.3 | 35 | 3.5 | 49 | 4.0 | 70
  +1 2,4-Dinitrophenol: 8 mg/kg, i.v. (2 mg/ml solution)
  0-30 1.8 | 0.77 | 2.8 | 35 | 6.2 | 28 | 4.4 | 34
  30-60 0.6 | 0.85 | 3.0 | 36 | 5.6 | 28 | 3.7 | 41
  60-90 0.3 | 0.79 | 3.1 | 34 | 4.1 | 25 | 4.6 | 59
  90-120 0.6 | 0.80 | 3.6 | 32 | 4.1 | 22 | 5.4 | 61
  120-150 0.6 | 0.91 | 3.8 | 30 | 3.8 | 24 | 5.8 | 72

Experiment 30, chicken 13, weight 1.5 kg

-90 Intracrop infusion: water 1 ml/min
  I.v. infusion: PAH 8 g/l, inulin 6 g/l, (0.5 ml/min)
  (-30)-(-15) 0.6 | 1.24 | 4.1 | 37 | 4.4 | 30 | 5.2 | 59
  (-15)-(0) 0.9 | 1.29 | 4.3 | 39 | 4.4 | 30 | 5.2 | 63
  +1 2,4-Dinitrophenol: 6 mg/kg, i.v. (2 mg/ml solution)
  0-30 1.1 | 0.92 | 4.0 | 28 | 6.8 | 23 | 6.9 | 31
  30-60 0.8 | 0.73 | 3.7 | 40 | 6.5 | 20 | 4.7 | 35
  60-90 0.8 | 0.88 | 4.0 | 42 | 5.9 | 22 | 4.8 | 42
  90-120 0.8 | 1.01 | 4.8 | 35 | 5.5 | 21 | 6.4 | 47
  120-150 0.8 | 1.95 | 6.2 | 36 | 5.6 | 31 | 5.6 | 40

Pyrazinamide. Since pyrazinamide in oral doses of 1 to 3 g decreases uric acid clearance in man (Yu et al., 1957), we have tested this compound in chickens in the hope that the results might throw some light on the question of whether the effect in man is due to inhibition of secretion or facilitation of reabsorption. Pyrazinamide in intravenous doses of 50 to 100 mg/kg was found to have no effect on uric acid clearance in four birds. (Experiments not shown.) In higher doses there was some depression in secretion of uric acid along with a marked depression of GFR and some manifestations of systemic toxicity.

Discussion. As both probenecid and DNP reduced urate and PAH urinary output, we have considered the possibility that uric acid secretion, in this species, shares the common mechanism for that of organic acids. Uric acid also depresses the ability of rabbit kidney to accumulate PAH without affecting the respiratory rate of the tissue (Despopoulos, 1957). Further evidence for competitive interference of PAH and uric acid for the common transport mechanism in the chicken was advanced by Berger et al. (1959). These investigators have reduced the clearance of urate by approximately 60% when PAH was infused at the rates close to maximum for tubular transport.

This presumed competition necessitated a further set of controls; all types of experiments presented here were repeated without PAH (not shown). It was found that the effects of the various drugs on uric acid excretion agreed with those in which PAH was infused. Perhaps the absolute excretion of uric acid may have been altered but in view of the variations among individual birds this was not detectable.

These observations indicate that a single intravenous injection of 100 to 120 mg/kg of probenecid is strikingly more effective in lowering
the clearance of uric acid than 200 mg/kg of sodium salicylate or 6 to 11 mg/kg of DNP under comparable experimental conditions.

In our experience, the reduction of PAH clearance by sodium salicylate (table 2) seems to be of only 30 to 60 minutes' duration. The results were not clear enough to postulate a different mechanism of action from probenecid. Salicylate, then, may also act on the common organic acid secreting mechanism for urate and PAH.

Two of the four DNP experiments showed no rise in plasma uric acid levels for 60 to 90 minutes with simultaneous drop in urinary excretion, as represented in experiment 30, table 3. In experiment 35 (the same table) there is a small but consistent rise in plasma urate.

Consequently, some of our data suggest that high energy phosphate bonds, generated in the process of aerobic oxidations, known to be uncoupled by DNP in mammalian kidney cells (Cross et al., 1949) may play a role in both synthesis and transport of uric acid by tubular epithelium.

That only partial effects were obtained with DNP may be attributed to incompleteness of inhibition of aerobic phosphorylation at the doses studied (Mudge and Taggart, 1950). By comparison, DNP inhibited PAH and phenol red secretion to about 50% in dogs (Mudge and Taggart, 1950). To almost the same degree, uric acid and PAH secretion have been inhibited in our study. Taggart and Forster (1950) showed that DNP can totally inhibit transport at least of phenol red in the isolated tubules of the flounder, while only partial inhibition was evident with lesser concentrations of DNP.

The administration of 100 mg/kg of pyrazinamide had no effect on uric acid secretion. This would suggest that uric acid retention in man following this drug is due to enhanced reabsorption, a process probably absent in the bird.

SUMMARY

Probenecid was found to diminish renal tubular secretion of uric acid and to raise markedly plasma urate concentrations in the chicken.

Sodium salicylate and 2,4-dinitrophenol inhibition were less effective, but also reduced uric acid excretion. The 2,4-dinitrophenol effect suggests that the tubular transport of uric acid may be dependent on energy supplied by oxidative phosphorylation.

Since probenecid and sodium salicylate have previously been shown to inhibit reabsorption of uric acid in mammals, it is possible that there is a common factor in the transport mechanism for reabsorption and secretion of this substance.

Pyrazinamide was without effect on uric acid clearance in this species.

ACKNOWLEDGMENT. We wish to thank Dr. E. K. Marshall, Jr., and Dr. Thomas H. Maren for advice during the course of this study and also for a critical review of the manuscript.

We are indebted to the Merck Institute for Therapeutic Research for a supply of probenecid and to the American Cyanamid Company for samples of pyrazinamide.

REFERENCES