EFFECT OF RENAL BETA- AND ALPHA-
ADRENERGIC STIMULATION ON PROXIMAL
SODIUM REABSORPTION IN DOGS

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SUMMARY

1. Micropuncture studies were performed in dogs to evaluate more directly the
suggestion from clearance experiments that alpha-adrenergic stimulation enhances
and beta-adrenergic stimulation depresses proximal sodium reabsorption. Experi-
ments were performed during unilateral renal artery infusion of the appropriate
drugs in the doses used in previous clearance studies.

2. To study pure beta stimulation, collections were made during alpha blockade
with phenoxybenzamine and re-collections during the addition of beta stimulation
with isoproterenol. No significant changes were noted in the ratio of inulin concentra-
tions in tubular fluid and plasma (TF/P)\textsubscript{in} (1.49 \pm 0.04 to 1.52 \pm 0.05), absolute sodium
reabsorption (23 \pm 1 to 23 \pm 3 nl/min), single nephron glomerular filtration rate
(SNGFR) (75 \pm 7 to 76 \pm 15 nl/min) and the ratio of SNGFR to inulin clearance
(SNGFR/C\textsubscript{in}) \times 10^6 (2.95 \pm 0.4 to 2.65 \pm 0.4).

3. To study pure alpha-adrenergic stimulation, collections were made during beta
blockade with propranolol and again during the addition of alpha-adrenergic
stimulation with nor-adrenaline. There were no significant changes in (TF/P)\textsubscript{in}
(1.50 \pm 0.09 to 1.42 \pm 0.04), absolute sodium reabsorption (25 \pm 7 to 17 \pm 4 nl/min),
SNGFR (68 \pm 13 to 58 \pm 10 nl/min) or SNGFR/C\textsubscript{in} \times 10^6 (2.76 \pm 0.6 to
2.51 \pm 0.5).

4. C\textsubscript{in} increased slightly after beta but not after alpha stimulations. \textit{p}-Amino-
hippuric acid clearance (C\textsubscript{PAH}) and urine sodium excretion (U_{NaV}) were not signifi-
cantly different in either set of studies.

5. We conclude that neither alpha nor beta adrenergic stimulation has a significant
effect on proximal sodium reabsorption when infused in doses that do not alter renal
haemodynamics.

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In recent studies (Gill & Casper, 1971a, b, c; Gill, Tate & Kelly, 1971) Gill and associates have presented evidence suggesting an important regulatory role for adrenergic mediators in the control of proximal tubular sodium reabsorption. In clearance experiments in hypophysectomized dogs undergoing water diuresis, they found that beta-adrenergic stimulation decreased (Gill & Casper, 1971a), and alpha-adrenergic stimulation increased (Gill & Casper, 1971c), proximal sodium reabsorption. It was further shown that adenosine 3':5'-cyclic monophosphate (cyclic AMP) (Gill & Casper, 1971b) and cyclic GMP (Gill et al., 1971), produced effects similar to beta- and alpha-adrenergic stimulation, respectively. They suggested that alpha- and beta-adrenergic mediators had counterbalancing effects on proximal tubular sodium reabsorption, and that these effects were mediated at the cellular level by cyclic AMP and cyclic GMP.

Because of the potential importance of these observations, we have attempted to confirm Gill & Casper's (1971a, c) findings, by the more direct method of micropuncture. We were unable to find any significant direct effect of pure alpha- or beta-adrenergic stimulation on proximal tubular sodium reabsorption.

METHODS

Experiments were performed on hydropenic mongrel dogs weighing 13–27 kg. The techniques were similar to those described by Auld, Alexander & Levinsky (1971). The only additional procedure was that a size 26 needle, connected to a syringe by polyethylene tubing, was inserted in a retrograde direction into the main renal artery, usually at the division of the artery into its two main branches, and fixed with adhesive. The needle was kept open with an infusion of 0.15 M-NaCl at 0.5–1.0 ml/min.

Two series of experiments were performed.

Series I—Renal beta-adrenergic stimulation during alpha-adrenergic blockade

Alpha-adrenergic blockade was induced by the infusion of phenoxybenzamine (0.18 μg min⁻¹ kg⁻¹) in saline into the left artery at 0.5–1.0 ml/min. At least 20 min after the infusion had been started, initial tubular punctures and clearance collections were obtained. Then isoproterenol, 0.036 μg min⁻¹ kg⁻¹, was added to the renal artery infusate and continued in saline at 0.5–1.0 ml/min. After at least 20 min clearance and re-puncture collections were begun. In four of the six experiments re-punctures were alternated with collections from fresh tubules.

Series II—Renal alpha-adrenergic stimulation during beta-adrenergic blockade

Beta-adrenergic blockade was induced by the infusion of propranolol (0.17 μg min⁻¹ kg⁻¹) in saline into the left renal artery at 0.5 ml–1.0 ml/min. After at least 20 min initial tubular samples and clearance collections were obtained. Then noradrenaline (0.009 μg min⁻¹ kg⁻¹) was added to the renal artery infusate, which was continued at 0.5–1.0 ml/min. At least 20 min later tubular re-puncture and clearance collections were begun. In four of six experiments re-punctures were alternated with collections from fresh tubules.
Most tubular fluid samples were obtained from the latest proximal tubular segments available on the surface of the kidney; however, occasional re-punctures at earlier sites were not rigidly excluded. In both series values for single nephron glomerular filtration rate (SNGFR) reported are from freshly punctured tubules only. Mean values for both clearance and micro-punctured data were calculated for each experiment and values given in the text and Tables are means ± SE of the means of individual experiments. Statistical significance of the differences of the means was determined by Student’s t test, using paired analysis. The analytical methods have been described by Auld et al. (1971).

RESULTS

Effect of pure renal beta-adrenergic stimulation

The results from this series of experiments are summarized in Table 1. In six dogs twenty-eight tubules were punctured during renal alpha-adrenergic-blockage (phenoxybenzamine) and re-punctured during the addition of beta-adrenergic stimulation (isoproterenol). The (TF/P)IN ratio was not different, 1.49 ± 0.04 before and 1.52 ± 0.05 (P > 0.4) after pure beta stimulation. In four dogs SNGFR was 75 ± 7 nl/min (twenty-one tubules) before and 76 ± 15 nl/min after (eighteen tubules) pure beta stimulation. Absolute sodium reabsorption in the proximal tubule was not altered in these four dogs. CIN increased slightly. CFPAN and filtration fraction (CIN/CFPAN) did not change significantly. Sodium excretion remained negligible. SNGFR/CIN (10⁶) was 2.95 ± 0.4 before and 2.65 ± 0.41 (P > 0.2) after beta stimulation. In the right or control kidney (not shown in Table 1) CIN, CFPAN and UNaV were not significantly different from those of the experimental kidney. Changes in haematocrit, plasma protein and systemic blood pressure were not significant.

Effect of pure renal alpha-adrenergic stimulation

The results from this series of experiments are summarized in Table 2. In six dogs thirty tubules were punctured during renal beta-adrenergic blockade (propranolol) and re-punctured during the addition of alpha-adrenergic stimulation (noradrenaline). The (TF/P)IN ratio was not different, 1.50 ± 0.09 to 1.42 ± 0.04 (P > 0.1). In four dogs SNGFR was 68 ± 13 nl/min (twenty-four tubules) before and 58 ± 10 nl/min (twenty-one tubules) after pure alpha stimulation; the tendency of SNGFR to decrease was not statistically significant (P > 0.1). Absolute sodium reabsorption also tended to fall, but the change was not significant (P > 0.1). CIN, CFPAN, filtration fraction and sodium excretion in the experimental kidney were not significantly altered by alpha stimulation. The clearance values from the right or control kidney (not shown in Table 2) were not significantly different from those from the left kidney. SNGFR/CIN (10⁶) was 2.76 ± 0.60 before and 2.51 ± 0.52 after alpha stimulation (P > 0.1). Haematocrit, plasma protein and systemic blood pressure were not significantly altered.

DISCUSSION

In contrast to the experiments of Gill & Casper (1971a, c), our studies failed to demonstrate any effect of alpha- or beta-adrenergic mediators on proximal fractional sodium reabsorption. In agreement with their conclusions, we found no significant effect of either type of agent in the doses they used on renal plasma flow or filtration fraction. GFR was unaffected by alpha
<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>(TF/P)Ia</th>
<th>SNGFR (nl/min)</th>
<th>Absolute water reabsorption (nl/min)</th>
<th>CIn (ml/min)</th>
<th>CPAH (ml/min)</th>
<th>CIn/CPAH</th>
<th>UNaV (μEq/min)</th>
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<td>Mean ± SE</td>
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<td>1:52†</td>
<td>75</td>
<td>76†</td>
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<td>23:4†</td>
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Abbreviations: C, control, left renal artery infusion of phenoxybenzamine; E, experimental, addition of isoproterenol to left renal artery infusion; (TF/P)Ia, tubular fluid to plasma concentration ratio of inulin; SNGFR, single nephron glomerular filtration rate; CIn, clearance of inulin, CPAH, clearance of PAH; UNaV, urinary sodium excretion. * P < 0.05. †Not significantly different.
<table>
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<tr>
<th>Expt. No.</th>
<th>Absolute water reabsorption (ml/min)</th>
<th>SNGFR (ml/min)</th>
<th>(TF/P)_{ha}</th>
<th>C_16, C_16/MA</th>
<th>U_{Na}, V (mEq/min)</th>
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<td>Mean±S.E.</td>
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<td>1.63 ± 0.10</td>
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**Abbreviations** are the same as Table 1. C = Control, left renal artery infusion of propranolol; E = experimental, addition of norepinephrine to left renal artery infusion. † Not significantly different.
mediators, but increased slightly in response to beta stimulation. SNGFR did not change significantly during the action of either mediator, although there was some tendency for it to decrease during alpha stimulation. Absolute proximal reabsorption was not altered significantly by either agent, although there was a downward trend during alpha stimulation, proportionate to the similar trend in SNGFR.

As far as possible, we followed the protocol of Gill & Casper (1971a, c) exactly. The mediators used, the rate and locus of administration, and the time sequence of observations were identical. The only major difference is that they studied hypophysectomized dogs undergoing a water diuresis, while we used intact hydropenic dogs. Although vasopressin does not appear to alter proximal reabsorption directly (Davis, Knox & Berliner, 1967), it is conceivable that vasopressin interferes with a proximal action of the adrenergic mediators. It is also possible that prior volume expansion (by water in the clearance studies) is necessary for a proximal tubular action of the adrenergic mediators. There is no evidence to support either of these possibilities, however.

\[ \frac{V}{100 \text{ ml GFR}} \] (where \( V \) is the volume of urine in ml/min) was used as the index of distal delivery of filtered sodium and water in the clearance studies of Gill & Casper (1971a, c). In absolute terms, this quantity increased by 3.7 ml/100 ml GFR during beta stimulation (Gill & Casper, 1971a) and decreased by 1.5 ml/100 ml of GFR during alpha stimulation (Gill & Casper, 1971c). If these values are interpreted as indices of the absolute magnitude of changes in proximal reabsorption, it would be most unlikely that such changes could be detected by the re-collection micropuncture technique. However, it is highly improbable that \( \frac{V}{100 \text{ ml of GFR}} \) is a quantitatively exact index of distal delivery (Levinsky & Levy, 1972). Hence it seems more reasonable to interpret a change in \( \frac{V}{100 \text{ ml of GFR}} \) as a fraction of the control value rather than as an index of the absolute magnitude of a change in proximal fractional reabsorption. As a percentage of control, \( \frac{V}{100 \text{ ml of GFR}} \) increased about 40% during beta stimulation and decreased about 20% during alpha stimulation in the experiments of Gill & Casper (1971a, c). They interpreted these percentage changes as indices of comparable changes in proximal fractional reabsorption. Changes of this magnitude, comparable to those found during extracellular volume expansion with saline or colloids, have been detected readily in our own laboratory (Auld et al., 1971) as well as by others (Howards, Davis, Knox, Wright & Berliner, 1968; Dirks, Cirksena & Berliner, 1965; Mandin, Israelit, Rector & Seldin, 1971). Therefore, we conclude that alpha- and beta-adrenergic mediators do not alter proximal reabsorption to the major degree proposed by Gill & Casper (1971a, c). We cannot eliminate the possibility that smaller changes in fractional reabsorption, of the order of 10% or less, can be induced by these agents.

We cannot rule out completely the possibility that the clearance experiments reflect a change in sodium reabsorption only in deep nephrons not available for micropuncture. However, Gill & Casper (1971a, c) did not find any change in \( p \)-aminohippuric acid clearance or extraction. Our results reveal no change in renal plasma flow nor in distribution of filtrate among nephrons (SNGFR/C\( _{\text{In}} \)). Therefore, this possibility seems unlikely. We also considered the possibility that the infusion of drugs into the renal artery was unequally distributed throughout the kidney and did not reach the nephrons which were punctured. To eliminate this potential error, in most experiments a small injection of Lissamine Green (\(<0.1 \text{ ml of a } 10\% \text{ solution}\)) was made through the renal artery needle which delivered the drug infusions. The visible area of the kidney surface was always uniformly perfused and all tubules punctured demonstrated filtration
of Lissamine Green. We believe, therefore, that the nephrons tested by micropuncture were adequately perfused with the appropriate drugs.

Recent publications (Schrier, Lieberman & Ufferman, 1972; Klein, Liberman, Laks & Kleeman, 1971; Levi, Grinblat & Kleeman, 1971; Handler, Bensinger & Orloff, 1968) have stressed the effect of adrenergic drugs on water reabsorption. If these drugs directly affect tubular water reabsorption, as suggested by studies in vitro (Handler et al., 1968) and in vivo (Schrier et al., 1972; Klein et al., 1971; Levi et al., 1971), then interpretation of clearance experiments dependent upon constant water permeability might not be valid. A decrease in volume and free water clearance (CH\(_{2}O\)) would be anticipated with isoproterenol and an increase after noradrenaline. These effects would be interpreted as an increase in proximal sodium reabsorption after beta stimulation and a decrease after alpha stimulation when, in fact, only distal water permeability had been altered. This is, however, just the opposite of what was found by Gill & Casper (1971a, c). Moreover, Schrier et al. (1972) have suggested that isoproterenol acts by stimulation of the hypothalamoneurohypophyseal system, so has no antidiuretic effect when infused in small amounts directly into the renal artery. From these considerations, it would appear unlikely that the discrepancy between the clearance and micropuncture results can be explained by interference with proper interpretation of clearance results due to adrenergic effects on distal tubular permeability. Nevertheless, it would seem desirable to interpret clearance indices of tubular reabsorption very cautiously in studying adrenergic agents, until possible effects of these mediators on factors such as distal permeability and medullary blood flow have been more completely clarified.

Our experiments do not confirm or negate the proposal of Gill & Casper (1971b) and Agus, Puschett, Senesky & Goldberg (1971) that cyclic AMP depresses tubular sodium reabsorption nor the recent suggestion (Gill et al., 1971) that cyclic GMP enhances proximal reabsorption. Our data also have no direct bearing on the proposal that beta mediators exert their renal tubular effects via cyclic AMP and alpha mediators via cyclic GMP. Our results do indicate, however, that acute changes in proximal tubular sodium reabsorption either do not occur in response to these mechanisms, or are of relatively small magnitude.

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