# Effect of Adrenergic Blocking Agents on the Vasopressin Inhibiting Action of Norepinephrine[1] (34424)

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Norepinephrine increases urine flow rate and free water clearance without altering glomerular filtration rate in almost all human subjects (1, 2). Baldwin *et al.* (2) found that this effect was most marked in subjects who started with a negative free water clearance and presumably, therefore, with elevated levels of antidiuretic hormone (ADH). Fisher (3) showed that the action of exogenous ADH given to water loaded subjects could be inhibited by norepinephrine. Alpha adrenergic drugs cause diuresis and beta adrenergic drugs cause antidiuresis in the normally hydrated rat (4). We studied the influence of alpha- and beta-adrenergic blocking agents on the norepinephrine inhibition of ADH in water loaded rats receiving exogenous ADH.

*Materials and Methods.* Albino Sprague-Dawley rats, weighing 100–200 g, were used. Ethyl alcohol anesthesia and water diuresis were induced as previously described (5). A tail vein was used for all iv infusions and the bladder tube technique was used for collecting urine specimens. Dextrose (2.5%) in water was infused until a constant urine volume was achieved. Sequential 60-min infusions were given as follows: (i) 2.5% dextrose in water; (ii) ADH[4], 0.3 mU/hr; (iii) ADH plus norepinephrine[5], 10.0 μg/hr; and (iv) ADH, norepinephrine and either the alpha-adrenergic blocking agent, phenoxybenzamine,[6] 60 μg/hr, or the beta-adrenergic blocking agent, propranolol[7], 30 μg/hr. All infusions were given in 2.5% dextrose. During each 60-min infusion, three 20-min urine collections were made. Although the first 20 min period if any infusion did show changes in the measured parameters, these were not always significant and are not shown in the tables. These periods do appear in the figures. Urinary output was measured directly. Sodium and potassium were determined by flame photometry and urine osmolality by freezing point depression. Because of its extremely rapid rate of diffusion across all body cells, ethanol attains the same concentration in blood, intracellular and intraluminal water. Therefore, in the present experiments it contributed to the urinary osmolality in a concentration equal to its level in plasma. As the latter was maintained relatively constant by the ethanol infusion, absolute changes in urine osmolality were probably minimally affected and no corrections for urinary ethanol content were necessary. The relatively high urinary osmolality observed in these rats during maximal water diuresis is a reflection of the added contribution of the ethanol.

*Results. Urine Volume* (see Table I and Fig. 1). ADH infusion decreased the 20-min urine volume from an average control value of 1.75 to 0.52 ml ($p < 0.001$) and 0.43 ml ($p < 0.001$) in consecutive periods. The addition of norepinephrine reversed this effect with urine volumes rising to 1.25 and 1.39 ml. Compared to the ADH infusion period these. values are significantly different, ($p < 0.05$ and $p < 0.001$, respectively) and they did not differ significantly from the control periods. The addition of phenoxybenzamine

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[5] Levophed, Winthrop.

[6] Dibenzilyne, CIBA.

[7] Inderal, Ayerst.

TABLE I. Effects of ADH, Norepinephrine, and Adrenergic Blocking Agents on Urine Volume (ml/20 min).

| | | ADH | | Norepinephrine | | Phenoxybenzamine | | Propranolol | |
|---|---|---|---|---|---|---|---|---|---|
| Rat | Control[a] | II | III | II | III | II | III | II | III |
| 1 | 0.9 | 0.2 | 0.3 | 1.4 | 1.0 | — | — | — | — |
| 2 | 0.9 | 0.3 | 0.4 | 1.0 | 1.0 | — | — | — | — |
| 3 | 1.3 | 0.3 | 0.2 | 1.1 | 1.5 | — | — | — | — |
| 4 | 1.7 | 0.1 | 0.1 | 0.6 | 1.6 | — | — | — | — |
| 5 | 1.0 | 0.5 | 0.2 | 0.7 | 1.0 | 0.5 | 0.6 | — | — |
| 6 | 2.4 | 0.6 | 1.0 | 2.3 | 2.3 | 0.7 | 0.2 | | |
| 7 | 2.9 | 0.8 | 0.5 | 0.8 | 1.2 | 0.3 | 0.1 | — | — |
| 8 | 1.0 | 0.3 | 0.3 | 0.9 | 0.9 | — | — | 2.0 | 1.3 |
| 9 | 3.5 | 1.2 | 1.0 | 2.6 | 1.4 | — | — | 1.6 | 2.0 |
| 10 | 2.0 | 0.9 | 0.3 | 1.1 | 2.0 | — | — | 1.6 | 2.0 |
| Mean | 1.8 | 0.5 | 0.4 | 1.25 | 1.4 | 0.5 | 0.3 | 1.7 | 1.8 |
| SD | 0.86 | 0.33 | 0.30 | 0.64 | 0.43 | 0.14 | 0.20 | 0.17 | 0.31 |

[a] Average of three 20-min control peroids.

caused the urine volume to fall again ($p<$ 0.001) in both the second and third periods to levels not significantly different but perhaps even lower than those from the ADH alone periods. Propranolol had no remarkable effect; the urine volumes continued to rise toward control values.

*Urine osmolality* (see Table II and Fig. 1). ADH infusion increased the urine osmolality from an average control value of 286 to 410 mOsm/kg ($p<0.001$) and 536 mOsm/kg ($p<0.001$) in consecutive periods. Norepinephrine caused the urine osmolalities to decrease to 407 and 322 mOsm/kg in the second and third periods, respectively. Compared to the periods when ADH was infused alone these values are significantly different (both $p < 0.001$) while not differing significantly from the control periods. The addition of phenoxybenzamine caused an increase in the osmolality to 401 mOsm/kg ($p<0.05$) in the third period. Propranolol had no remarkable effect; the urine osmolality continued dropping toward control values.

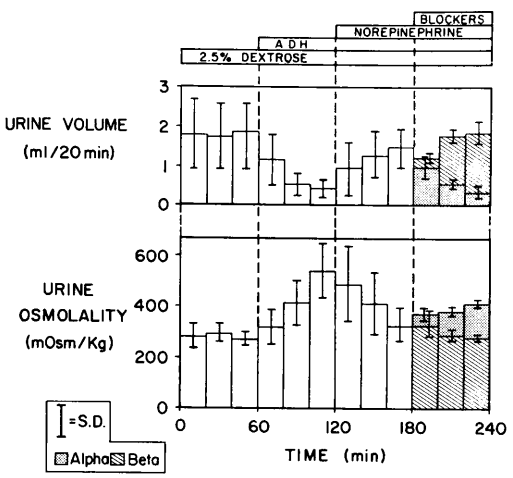


FIG. 1. The mean urine volume and osmolality of each collection period is shown. The content of the infusate is labeled at the top of the figure. For example, in the period 180–240 min, the rats were getting 2.5% dextrose, ADH, norepinephrine, and either an alpha- or beta-adrenergic blocking agent.

*Urine sodium* (see Table III). During the periods of ADH-induced antidiuresis a marked reduction of sodium excretion was observed in each rat. During norepinephrine infusion the effect was variable, sodium excretion dropped further in 4/6 rats and rose in 2/6 rats. During the phenoxybenzamine periods a more severe reduction in sodium output was observed than with either ADH alone or ADH plus norepinephrine. Propranolol seemed to have a biphasic effect, producing a rise in sodium excretion early and a fall later, and like phenoxybenzamine, it produced lower sodium output values than either ADH alone or ADH plus norepinephrine.

*Urine potassium* (see Table IV). During ADH-induced antidiuresis, a marked reduction of potassium excretion occurred in each

TABLE II. Effects of ADH, Norepinephrine, and Adrenergic Blocking Agents on Urine Osmolality (mOsm/kg of $H_2O$).

| | | ADH | | Norepinephrine | | Phenoxybenzamine | | Propranolol | |
|---|---|---|---|---|---|---|---|---|---|
| Rat | Control[a] | II | III | II | III | II | III | II | III |
| 5 | 269 | 335 | 500 | 470 | 315 | 355 | 385 | — | — |
| 6 | 310 | 571 | 525 | 290 | 270 | 390 | 420 | — | — |
| 7 | 312 | 372 | 514 | 509 | 429 | 373 | 400 | — | — |
| 8 | 288 | 463 | 600 | 558 | 383 | — | — | 270 | 270 |
| 9 | 253 | 330 | 356 | 217 | 227 | — | — | 283 | 270 |
| 10 | 288 | 390 | 722 | 400 | 309 | — | — | 310 | 282 |
| Mean | 287 | 410 | 536 | 407 | 322 | 372 | 401 | 287 | 274 |
| SD | 20.9 | 84.3 | 110 | 120 | 67 | 14 | 14 | 16 | 5 |

[a] Average of three 20-min periods.

rat. Norepinephrine reversed this ADH effect in 5/6 rats. Phenoxybenzamine caused the potassium output to fall again, and in 2/3 rats it fell further than when ADH alone was infused. The propranolol had no remarkable effect, the rising potassium output which started with norepinephrine continued.

*Discussion.* The mechanism by which norepinephrine increases the urine output is not known. The effect seems to be independent of changes in GFR, RPF, osmotic clearance, and sodium excretion (1–3). Selkurt *et al.* (6) showed an increased urine volume secondary to elevated renal arterial perfusion pressure, and suggested the effect was due to a washout of medullary palilla sodium with a loss of the papillary osmotic gradient and an inability to concentrate urine despite the action of ADH on the collecting ducts. Although blood pressure was not recorded in the experiments reported here, it is possible that the norepinephrine may have elevated it and caused such a "pressure diuresis." This does not seem likely however, since pressure diuresis is associated with a marked increase in sodium clearance while in the present study the sodium excretion dropped in 4 of 6 rats. Furthermore, increased renal arterial pressure *per se* is probably functionally different from norepinephrine-induced pressure increase since the latter is associated with constriction of the vasa recti (7), an effect which tends to preserve the medullary osmotic gradient. The promptness of the antiduretic response to phenoxybenzamine in the current studies also suggests that medullary washout is not the mechanism of the norephinephrine effect. Finally, in unpublished work currently in progress in our laboratory we find the same norepinephrine inhibition of ADH in the absence of any significant hemodynamic alterations.

Fisher (3) reversed the antidiuretic effect of low but not high doses of exogenous ADH

TABLE III. Effects of ADH, Norepinephrine, and Adrenergic Blocking Agents on Urine Sodium Excretion ($\mu$eq/20 min).

| | | ADH | | Norepinephrine | | Phenoxybenzamine | | Propranolol | |
|---|---|---|---|---|---|---|---|---|---|
| Rat | Control[a] | II | III | II | III | II | III | II | III |
| 5 | 24.1 | 7.5 | 4.0 | 31.5 | 30.0 | 12.5 | 9.3 | — | — |
| 6 | 81.1 | 51.0 | 63.0 | 66.7 | 72.5 | 29.4 | 7.0 | — | — |
| 7 | 65.3 | 24.8 | 20.5 | 20.0 | 19.2 | 3.0 | 1.0 | — | — |
| 8 | 31.5 | 15.8 | 15.8 | 28.7 | 11.3 | — | — | 20.0 | 10.4 |
| 9 | 91.8 | 40.8 | 39.0 | 39.0 | 20.3 | — | — | 37.6 | 3.5 |
| 10 | 29.5 | 13.6 | 13.4 | 17.7 | 10.0 | — | — | 6.4 | 8.0 |

[a] Average of three 20-min periods.

TABLE IV. Effect of ADH, Norepinephrine, and Adrenergic Blocking Agents on Urine Potassium Excretion ($\mu$eq/20 min).

| | | ADH | | Norepinephrine | | Phenoxybenzamine | | Propranolol | |
|---|---|---|---|---|---|---|---|---|---|
| Rat | Control[a] | II | III | II | III | II | III | II | III |
| 5 | 18.8 | 15.5 | 6.0 | 35.0 | 24.0 | 15.0 | 13.2 | — | — |
| 6 | 50.8 | 15.6 | 25.1 | 37.3 | 34.5 | 22.6 | 2.0 | — | — |
| 7 | 47.8 | 13.6 | 15.5 | 21.6 | 27.2 | 8.5 | 1.5 | — | — |
| 8 | 15.1 | 6.3 | 6.3 | 22.5 | 24.8 | — | — | 40.4 | 32.7 |
| 9 | 49.1 | 27.4 | 25.1 | 11.7 | 7.0 | — | — | 13.6 | 12.2 |
| 10 | 17.5 | 5.6 | 5.6 | 9.8 | 11.6 | — | — | 9.0 | 12.6 |

[a] Average of three 20-min periods.

with norepinephrine. Direct antagonism of ADH action by norepinephrine *in vitro* was shown by Handler *et al.* (8) using the isolated toad bladder. It is, therefore, suggested that a competitive mechanism between norepinephrine and ADH exists at the renal tubular level.

Infusion of the alpha-adrenergic blocking agent, phenoxybenzamine, completely reversed the "diuretic" effect of norepinephrine and allowed the antiduretic effect of ADH to reappear. The beta-adrenergic blocking agent, propranolol, did not reverse the action of norepinephrine; in fact, it may have mildly augmented it. These observations suggest that norepinephrine inhibits the antidiuretic effect of ADH by acting as an alpha-adrenergic agent, probably through an alpha receptor in the kidney.

It was shown that ADH caused an increase in renal medullary adenylcyclase (9) and that cyclic AMP mediated ADH action (10). Since alpha- and beta-adrenergic activity affect the cyclic AMP concentration in many tissues (11–13), it is tempting to speculate that norepinephrine (an alpha-adrenergic agent) inhibits ADH antidiuresis by antagonizing adenylcylase activation and reducing the concentration of cyclic AMP. Phenoxybenzamine, an alpha-adrenergic blocking agent, may simply prevent this effect. Adenylcylase has been likened to a "beta-adrenergic receptor site" (14), but we are unable to find data showing an ADH-like effect of beta-adrenergic agents. It, therefore, seems premature to speculate about the possibility that ADH works through beta-adrenergic receptors in the kidney tubule.

*Summary.* The effects of phenoxybenzamine and propranolol on norepinephrine anti-ADH action were studied in water loaded rats. Norepinephrine blocked the ADH antidiuresis. This norepinephrine effect was blocked by phenoxybenzamine but not by propranolol. These observations suggest that different adrenergic receptors may have differing functions in the kidney with the alpha receptors supporting an anti-ADH effect.

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