Influence of adrenal cortex on body water distribution in rats

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Body water distribution was determined in rats by the dilution of serum albumin-1311 and Br82 and by desiccation. Methylprednisolone (MP) increased the plasma and intracellular compartments and decreased the interstitial volume in intact hydrated rats. The body water moved in the opposite direction in the adrenalectomized, salt-maintained rats, and MP prevented this movement in terms of intracellular and interstitial volumes. Adrenalectomized rats which were salt depleted had an increased intracellular volume with water coming from both extracellular compartments, and MP had no effect in this group. The effect of deoxycorticosterone acetate was to increase plasma volume in the intact hydrated and in both adrenalectomized groups. The relationship between these changes in body water distribution, hematocrit, and plasma osmolality and sodium concentrations has been discussed.

METHODS

The distribution of body water was determined in male Holtzman rats weighing 200–250 g. The animals were divided into three experimental groups referred to as: 1) intact, hydrated; 2) adrenalectomized, salt; and 3) adrenalectomized, no salt. All animals were first kept for at least 2 weeks in a quiet, temperature-controlled room. Adrenalectomy was accomplished through the lumbar approach under ether anesthesia. From the time of adrenalectomy until body fluid spaces were determined 1 week later, the adrenalectomized, salt group drank isotonic saline ad lib. and was given the same rat, mouse, GLF diet as the intact rats. The adrenalectomized, no salt group was maintained in the same manner as the adrenalectomized, salt group for 5 days postoperatively but was then placed on a sodium-deficient test diet (Nutritional Biochemicals Corp.) and distilled water for the last 48 hr. Methylprednisolone (MP) (Depo-Medrol, Upjohn) was injected intramuscularly in the daily dose of 1 mg/kg for 2 days in the hydrated, intact rats, and for 7 days in the adrenalectomized rats. DOC (deoxycorticosterone acetate, Percorten acetate, Ciba) was injected intramuscularly in the daily dose of 0.5 mg for the same period of time as the methylprednisolone.

Plasma volume was determined by the dilution of intravenously injected serum albumin-1311 (RISA) at 10 min. The extracellular space was simultaneously determined by the distribution of Br82 administered intraperitoneally with a correction for urinary excretion during the 2-hr period of distribution.

The Br82 space = net counts per second of Br82 retained X .93 X .95/(net counts per second of Br82 per milliliter plasma). The .93 represents water content of plasma and the .95 is the Donnan Gibb's factor. The quantitation of Br82 and 1311 in each plasma specimen was by the method of differential decay, the counting being performed with a well-type scintillation spectrometer. The interstitial volume was calculated by subtracting the plasma volume from the Br82 space. Total body water was determined by desiccation in an oven at 105 C until a constant body weight was attained. Intracellular volume was calculated by subtracting the Br82 space from the total body water. The degree of penetration of the Br82 into the erythrocytes was determined by comparing net counts per second per volume of plasma with the net counts per second per
equal volume of erythrocytes after the latter had been washed three times in isotonic saline. Plasma osmolality was determined by cryoscopy using the Advanced Instrument osmometer, and plasma sodium was determined by flame photometry. Statistical comparison of results was made using Student's t test for independent variables.

RESULTS

The data in Fig. 1 indicate that the glucocorticoid methylprednisolone (Medrol), significantly alters the volume of all three fluid compartments in the intact, hydrated rat, the fluid leaving the interstitial space and entering both the plasma and intracellular spaces. The changes after adrenalectomy when the rats were not salt depleted were in the opposite direction, with the fluid accumulating in the interstitial space from the plasma and intracellular spaces. The interstitial and intracellular volumes were restored to normal in these rats by the administration of methylprednisolone. The adrenalectomized rats which were allowed to develop salt depletion had a marked increase in intracellular volume at the expense of the other two compartments. Under these conditions methylprednisolone had no effect on body water distribution. Under all three experimental conditions the only effect of DOC was to increase plasma volume.

The mean ratio of erythrocyte-to-plasma Br- at the time of sacrifice ranged from .010 for the adrenalectomized, salt-maintained, methylprednisolone-treated group to .044 for the intact, hydrated, untreated group (Table 1).

The data in Table 2 indicate that there is a marked increase in hematocrit and a marked decrease in plasma osmolality and plasma sodium concentration in the untreated, adrenalectomized, salt-depleted rats, and these changes are prevented by the administration of DOC. There are similar but less marked changes effected by DOC in the other two groups. Methylprednisolone did not significantly increase the hematocrit in any of the experimental groups, and aside from an increase in plasma osmolality in the intact, hydrated rats, methylprednisolone did not alter plasma osmolality or sodium concentration.

DISCUSSION

The administration of the glucocorticoid, methylprednisolone, to intact, hydrated rats caused a redistribution of body water, the effect of which was to decrease interstitial volume with the water moving into the plasma and cells. The opposite effect, movement of water out of the plasma and intracellular compartment and into the interstitial volume, was seen in the adrenalectomized rats which were not salt depleted. The injection of methylprednisolone into these adrenalectomized animals prevented the redistribution of body water except in terms of plasma volume. As long ago as 1936, Harrop (5) described the evidence for a disturbance of capillary permeability in adrenal insufficiency leading to escape of
TABLE 1. Mean ratios of erythrocyte-to-plasma Br\textsuperscript{82} 2 hr after injection of the isotope

<table>
<thead>
<tr>
<th></th>
<th>Intact, Hydrated</th>
<th>Adrenalectomized, Salt</th>
<th>Adrenalectomized, No Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bro &amp; DOC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>0.044 (10)</td>
<td>0.040 (10)</td>
<td>0.028 (9)</td>
</tr>
<tr>
<td>Medrol</td>
<td>0.043 (9)</td>
<td>0.027 (10)</td>
<td>0.027 (9)</td>
</tr>
</tbody>
</table>

TABLE 2. Effect of MP and DOC on hematocrit, plasma osmolality, and plasma Na concentration in intact, hydrated rats, adrenalectomized rats given salt, and adrenalectomized, salt-depleted rats

<table>
<thead>
<tr>
<th></th>
<th>Hematocrit</th>
<th>Plasma Osmolality</th>
<th>Plasma Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact, hydrated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>30.6±0.6</td>
<td>300.6±0.8</td>
<td>145.0±1.9</td>
</tr>
<tr>
<td>MP</td>
<td>30.5±0.6</td>
<td>290.5±0.8</td>
<td>142.8±1.5</td>
</tr>
<tr>
<td>DOC</td>
<td>37.5±0.6</td>
<td>300.0±0.3</td>
<td>144.2±1.0</td>
</tr>
</tbody>
</table>

Adrenalectomized, salt

|                      |            |                   |               |
| No treatment         | 44.9±1.5  | 302.8±0.9         | 160.0±0.5*    |
| MP                   | 44.9±0.8  | 305.2±0.9         | 160.0±0.8     |
| DOC                  | 40.2±1.1  | 307.8±0.8         | 143.7±0.7†    |

Adrenalectomized, no salt

|                      |            |                   |               |
| No treatment         | 49.9±2.4  | 269.8±0.9         | 123.2±2.9*    |
| MP                   | 53.5±1.9  | 273.0±1.0         | 126.7±4.9     |
| DOC                  | 40.1±0.8† | 295.1±0.7         | 145.2±0.9†    |

Each value represents the mean ± SE. * Different from intact, hydrated, no treatment, P < .05. † Different from adrenalectomized, salt or adrenalectomized, no salt, no treatment, P < .05.

fluid from the vascular to the interstitial spaces. Gaudino and Levitt (4) have reported that adrenocortical extract increases the intracellular volume in normal dogs, and Streiten et al. have noted that there is an enlargement of normal human erythrocytes in vivo after the administration of hydrocortisone (personal communication).

Since it is extremely doubtful that an osmotic gradient exists across the cell membrane (10), the action of glucocorticoids on enlarging the intracellular compartment in the absence of salt depletion is most likely associated with an increase in the number of intracellular osmotically active particles. There are several possible mechanisms by which this might occur, and which would be accompanied by a passive movement of water into the cell. Glucocorticoids might cause an increased ion content of the cell resulting from an enhanced influx of ions since hydrocortisone has been found to enhance radioisodium influx into erythrocytes of adrenalectomized dogs in vitro (manuscript of D. H. P. Streiten and A. M. Moses, in preparation). Glucocorticoids might conceivably increase the osmotic coefficient of intracellular particles. The latter possibility might result from a liberation of bound intracellular cation or to a partial breakdown of large molecules, i.e., proteins. The opposite action resulting in a decrease in the number of intracellular osmotically active particles would lead to a decrease in cellular water content in the adrenal-insufficient, salt-maintained group. In the adrenalectomized rats which were allowed to become salt depleted, the extracellular hypotonicity was apparently the overriding factor, with the resultant osmotic gradient causing water to move into the cells. Under these conditions methylprednisolone had no further influence on expanding an already enlarged intracellular volume.

Hemoconcentration has been long recognized in adrenal insufficiency (5). Swingle et al. (16) reported that the hemoconcentration and sensitivity of the adrenalectomized dog to stress are related to the passage of water into cells, and that the beneficial effect of the glucocorticoid 2-methyl-α-fluorohydrocortisone in the adrenal-insufficient dog is due to a shift of fluid and electrolytes from intra- to extracellular compartments (15). Swingle’s conclusions were based on hemoconcentration (hematocrit) changes and plasma volume determinations but did not include measurements of total body water or extracellular volume. The present experiments demonstrate that water movements in or out of the intracellular compartment are poorly reflected by hematocrit changes, at least in the rat, because of the variability in plasma volume under the different conditions. For example, adrenal insufficiency without salt depletion does not alter the hematocrit since water leaves both plasma and intracellular compartments and the ratio between cell volume and plasma volume is insignificantly changed. Significant hematocrit changes have been found only under conditions where plasma and intracellular volumes do not change in parallel; i.e., where plasma volume alone significantly increased after the injection of DOC, or where water left the plasma and entered the cells in animals which were adrenalectomized and allowed to become salt depleted (Fig. 1, Table 2). In support of Swingle’s observations, other authors have claimed that glucocorticoids induce a movement of water out of the cell (or a decreased entry of water). During the development of adrenal insufficiency in the dog and man, there is a decrease in extracellular space with the water presumably moving into the cellular compartment (4, 6), and this abnormality is corrected in the human by hydrocortisone (6). However, the role of altered extracellular sodium concentration in these changes is not clear. The administration of a water load to adrenal-insufficient patients causes an excessive swelling of erythrocytes, and this excessive influx of water is prevented by pretreatment with cortisone (2). In contrast most of the water load in an adrenalectomized rat is retained in the extracellular fluid compartment (11). Frost and Talmage emphasized that a water load accumulated intracellularly in the adrenalectomized rat only when sodium depletion was present (3).

The effect of the mineralocorticoid deoxycorticosterone (DOC) on body water distribution in the intact, hydrated rats and in both groups of adrenalectomized animals was to increase the proportion of plasma water. DOC has previously been reported to decrease intracellular volume and expand extracellular volume in the
intact dog (1, 4), and to expand plasma volume in the adrenal-insufficient dog (17) and human (6). The movement of water into plasma is reflected in the decreased hematocrit in two of the three experimental groups treated with DOC (Table 2). The observed mineralocorticoid-induced retention of sodium and increased osmotic pressure of the plasma may be the basic mechanism involved. The effect of adrenal hormones on body water distribution therefore depends on the nature of the adrenal hormone, the metabolic state of the animal, including the degree of sodium depletion, the presence or absence of adrenal insufficiency, and perhaps the species being investigated.

A shift of water out of the cell into the extracellular space inhibits the release of vasopressin (13, 14). Therefore, the glucocorticoid-induced movement of water into the cell cannot directly explain the diuretic action of the glucocorticoids. If, however, the osmoreceptor cell is acting as a volume receptor, the shrinkage of which stimulates vasopressin release and enlargement of which inhibits vasopressin release, the hydrocortisone-induced movement of water into the cell would antagonize the effectiveness of an osmotic stimulus to vasopressin release and result in the necessity for a greater osmotic gradient (higher plasma osmolality) to be established before the osmoreceptor cell would shrink sufficiently to release vasopressin and cause an antidiuresis. This may be the explanation for the increase in plasma osmolality at which vasopressin is released in water-loaded subjects (8). In other words, steroids may impair movement of water out of the cell in response to an induced osmotic gradient and thereby delay the release of vasopressin. In in vitro experiments, hydrocortisone has been found to decrease the hematocrit changes which occur when erythrocytes are suspended in hypertonie saline (manuscript of D. H. P. Streten and A. M. Moses, in preparation).

Since the 2-hr Br\textsuperscript{2+} space is crucial to the present study of body water distribution, one might ask whether the methylprednisolone effect was related to an alteration of cellular permeability to Br\textsuperscript{2+}. This is probably not the case because 1) Br\textsuperscript{2+} is essentially confined to the extracellular space and in order to decrease this space and increase the intracellular space in the intact hydrated and adrenalectomized salt-maintained rats treated with methylprednisolone, the slight degree of cellular permeability would have to be significantly decreased; 2) in the methylprednisolone-treated intact hydrated group, the decrease in erythrocyte-to-plasma ratio of Br\textsuperscript{2+} was 0.1% and in the methylprednisolone-treated adrenalectomized salt-maintained group the ratio was decreased by only 1.7% which would not account for the decrease in extracellular space; and in the adrenalectomized, salt-maintained group in which the extracellular space was increased, there was decreased penetration of Br\textsuperscript{2+} into the erythrocytes to the extent of 1.7% (Table 1). The corollary possibility that increases in plasma volume might be more apparent than real because of a leakage of RISA from the vascular tree could not be directly investigated because of the inaccessibility of interstitial fluid. The possibility, although remote, does exist that methylprednisolone and DOC increase vascular permeability to RISA and that adrenalectomy decreases the plasma volume by significantly decreasing the slight permeability to RISA of normal blood vessels.

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REFERENCES