Changes in plasma vasopressin concentration and plasma osmolality in relation to age and time of day in the male Wistar rat

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The influence of age on several parameters related to water balance was studied in Wistar rats. Plasma AVP concentration and plasma osmolality were increased at midday in 21-month-old rats as compared with 3- and 4-month-old rats. Daily water intake per 100 g body weight was reduced in 14- and 21-month-old rats as compared with 3- and 4-month-old rats, but total water intake was unaltered. These results suggest that there is a change in water balance in Wistar rats with age. In order to obtain information about the influence of age on daily fluctuations in plasma AVP concentration and osmolality these parameters were determined in 4-month-old Wistar rats sacrificed at 2 h intervals during the day and in 20- and 31-month-old rats sacrificed at 8 h intervals. Plasma AVP concentrations were low during the light period and high during the dark period in 4-month-old rats. The relationship between plasma osmolality and plasma AVP concentration was dependent on the time of day in 4-month-old rats. Plasma AVP concentrations were higher at 16.00 than at 08.00 and 24.00 in 20-month-old rats, and higher at 24.00 than at 08.00 and 16.00 in 31-month-old rats. In contrast to the plasma AVP concentration during the light period, the average daily AVP concentration (average of plasma AVP concentrations at 08.00, 16.00 and 24.00) was increased in 31-month-old rats only. The relationship between plasma osmolality and plasma AVP concentration was not age-related. The results of the present study suggest that there is a circadian rhythm of plasma AVP concentration in Wistar rats which is age-related but which does not fully correlate with plasma osmolality.

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Reports about the effects of aging on parameters related to fluid balance in rats are inconsistent. For instance, unaltered as well as reduced concentrations of plasma AVP have been found in 30-month-old Fischer-144 rats (1, 2). In contrast, 32-month-old Wistar rats (3) and 20-23-month-old Long-Evans rats (4) show increased plasma AVP concentrations. The daily urinary excretion of AVP was found to be increased in 34-month-old Brown-Norway rats (5). Miller (4) found increases in urine production in 14- and 16-month-old Long-Evans rats, whereas other authors observed increases in urine production in rats at a more advanced age only, if at all (5–8). However, as these latter studies were performed in other strains of rats, inconsistencies among studies on the influence of aging on the fluid balance in rats may be largely strain-dependent. However, other potentially relevant factors have to do with a circannual rhythm of plasma AVP concentration (2), with the ages of the rats used and with their health status.

Data on the effect of aging on water balance in Wistar rats are scarce and have never been verified since the initial study of Fliers and Swaab (3). For instance, data on urine or plasma osmolality in relation to aging are not available for Wistar rats, and plasma AVP concentrations have not been determined in middle-aged animals. To date it is recognized that inclusion of middle-aged rats in aging studies is especially important (9, 10). Moreover, detailed data on several strains of rats are necessary to select a model that best fits the age-related changes in water homeostasis found in humans.

A further point of concern in studies on the influence of age on fluid balance is possible daily fluctuations in plasma AVP concentration and osmolality. It is not clear whether plasma AVP concentration shows a circadian rhythm in rats. Greeley et al. (11) reported that there is a circadian rhythm in plasma AVP concentrations in Sprague-Dawley rats, whereas Schwartz et al. (12) found that Long-Evans rats had a constant plasma AVP concentration throughout the day. Greeley et al. (11) suggested that the daily variation in plasma AVP concentration is regulated by corticosterone, but did not consider other factors, in particular osmolality. In view of the fact that age-related changes in circadian rhythms of hormones have been reported in man as well as in rats (13), changes in a circadian rhythm of plasma AVP concentration in relation to age seem possible.
Therefore, the present study was designed to obtain information on the influence of age and time of day on plasma AVP concentration and osmolality in rats. Two separate experiments were performed. The first experiment concerned the influence of age on plasma AVP concentration, plasma osmolality and water intake. The effects of age on daily fluctuations in plasma AVP concentration and osmolality were determined in the second experiment. The results from these experiments are deemed important for a better understanding of alterations in fluid balance with age.

Materials and methods

In the present study male Wistar rats [Bor-WISW(sPFCpb)] were used. They were supplied by Winkelmann (Borchen, FRG) at an age of two months and were kept under controlled environmental conditions (a light:dark cycle of 12 h light and 12 h darkness (lights on at 07.30), free access to food and water). The 50% survival age of the strain of rats used is 28 months. Animals were handled once a day in the two weeks preceding the experiments.

In the first experiment 60 rats were used, divided into four groups of 3-, 4-, 14- and 21-month-old animals. They were housed singly one month in advance of the experiment to allow determination of daily water intake. Water intake and body weights were determined daily in the two weeks preceding the experiment. Rats were sacrificed between 11.00 and 13.00.

In the second experiment 4-, 20- and 31-month-old rats were used. Groups of four animals of four months of age were sacrificed at 2 h intervals during the day. Groups of 5–6 animals of 20 or 31 months of age were killed by decapitation at 08.00, 16.00 and 24.00. Trunk blood was collected into chilled heparinized tubes and centrifuged at 1000×g for 30 min. Plasma samples were stored at −20°C until determination of plasma AVP concentration or kept on ice until determination of plasma osmolality within one hour.

Radioimmunoassay of AVP

Plasma AVP concentrations were determined in triplicates. AVP was extracted from plasma samples using heat activated Vycor® glass powder (Corning Glass Works, New York) (14). The recovery of 1 fmol [125I]AVP was about 75% with this method. Antiserum W1 used in this study was previously characterized before (15) and is directed at the C-terminus of the vasopressin molecule. As a diluent a Veronal/HSA buffer, pH 8.0, containing 20 mmol/l Veronal, 10 mmol/l EDTA, 155 mmol/l NaCl, 66 μmol/l cystine and 5 g/l HSA was used. To polystyrene tubes 50 μl appropriately diluted plasma extract, 25 μl tracer solution (1 fmol [125I-Tyr²]AVP, 2000 Ci/mmol, Amersham) and 25 μl 128×10⁴ times diluted antiserum were added. After 72 h of incubation, bound and free ligand were separated with Ficolldextran-coated charcoal (375 μg Ficol (Pharmacia), 375 μg dextran (Sigma) and 4 mg charcoal (Sigma) in 100 μl 50 mmol/l phosphate, pH 7.4; (16)). The assay was internally standardized. Internal standards were prepared from 1-ml portions of AVP-free plasma to which known amounts of AVP (Sigma) had been added. AVP-free plasma was obtained by pre-extraction with Vycor® glass powder. Displacement of tracer by extracted internal standards versus the amounts of AVP added to the portions of plasma served as an internal standard curve. The intra- and interassay coefficients of variation were 7.1% and 8.6%, respectively. Non-specific binding was <3% and binding of tracer by excess antibody was 90% with our assay. The detection limit was 0.2 fmol/tube.

Osmometry

Osmalities were determined in 50 μl freshly prepared plasma samples in triplicate by freezing-point depression, using a cryoscopic osmometer (Osmostat 030: Gonotec, Berlin, FRG). Special care was taken to follow the recommendations of Bevilacqua et al. (17). The intra-assay coefficient of variation of median values of samples measured in triplicate was 0.3%. The interassay coefficient of variation cannot be determined reliably in frozen plasma samples, since freezing has a variable effect on plasma osmolalities. The intra-assay coefficient of variation of median values of the standard measured in triplicate was 0.31%. The apparatus was carefully calibrated before use.

Statistics

A statistics package was used for statistical analysis (SAS, Cary). The data were subjected to a one factor (age

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Weight (g)</th>
<th>Plasma AVP concentration (pmol/l)</th>
<th>Plasma osmolality (mOsm/kg)</th>
<th>Water intake (ml/100 g body weight/24 h)</th>
<th>Total water intake (ml/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>272 ± 3</td>
<td>0.57 ± 0.12</td>
<td>293.5 ± 0.8</td>
<td>9.29 ± 0.26</td>
<td>25.1 ± 0.7</td>
</tr>
<tr>
<td>4</td>
<td>318 ± 9</td>
<td>0.33 ± 0.10</td>
<td>290.3 ± 0.7</td>
<td>7.98 ± 0.21</td>
<td>25.4 ± 0.7</td>
</tr>
<tr>
<td>14</td>
<td>465 ± 10</td>
<td>1.12 ± 0.18</td>
<td>291.8 ± 1.1</td>
<td>5.36 ± 0.16</td>
<td>24.9 ± 0.7</td>
</tr>
<tr>
<td>21</td>
<td>477 ± 13</td>
<td>2.57 ± 0.37</td>
<td>299.6 ± 1.2</td>
<td>5.64 ± 0.45</td>
<td>26.9 ± 2.1</td>
</tr>
</tbody>
</table>

Values with different superscripts differ significantly (p < 0.05). Values with identical superscript do not differ significantly.
or time of day) or two factor (age x time of day) analysis of variance (GLM procedure with correction for unequal cell sizes), followed by the Duncan-Waller test for post hoc multiple comparisons. When variances were non-homogeneous, rank scores were used for statistical analysis (18). When appropriate, data on plasma osmolalities and plasma AVP concentrations were analyzed using linear regression analysis and Pearson’s correlation test. Relationships between plasma osmolality and plasma AVP concentration were tested for parallelism and coincidence according to the general regression theory (19).

Results

In the first experiment it was found that the AVP concentration and osmolality of plasma collected between 11.00 and 13.00 were age-related (p-values <0.001, Table 1). The plasma AVP concentration was increased in 14- and 21-month-old animals compared with 3- and 4-month-old rats. Osmolality was increased in 21-month-old rats as compared to 3- and 4-month-old rats, and in 14- and 21-month-old rats as compared with 4-month-old rats. Daily water intake was age-dependent (p<0.001, Table 1): 4-month-old rats drank less than 3-month-old rats, and 14- and 20-month-old rats drank less than 3- and 4-month-old rats.

In the second experiment it was found that both plasma AVP concentration and osmolality changed during the day in 4-month-old rats (p-values <0.01, Fig. 1). The Duncan-Waller test revealed the following differences: the concentration of plasma AVP was lower at 08.00 and 10.00 than at all other time points and was higher at 24.00 than at 08.00 to 16.00. Osmolality was higher at 10.00 and 24.00 than at the other time points, except for 22.00. 02.00 and 08.00. Osmolality was lower at 06.00, 12.00 to 16.00 and 20.00 than at 22.00 to 02.00 and 08.00 to 10.00.

Furthermore, it was found in the second experiment that plasma AVP concentration was influenced by age and time of day (p-values <0.001 and <0.01, respectively; Fig. 2). In addition, there was a significant interaction between these variables (p<0.001). In 4- and 31-month-old rats, the highest AVP concentration was found at 24.00; in the 20-month-old rats at 16.00. Osmolality was also related to age and time of day (p-values <0.01 and <0.05, respectively; Fig. 3). Osmolality was highest at 24.00 in 4- and 31-month-old rats. Osmolality was not dependent on time of day in 20-month-old rats. The average daily plasma AVP concentration and osmolality (averages of data obtained with plasma of rats killed at 08.00, 16.00 and 24.00) were age-related (p-values <0.001, Table 2). The average daily AVP concentration was increased in 31-month-old rats as compared with 4- or 20-month-old rats. The average daily osmolality was increased in 20- and 31-month-old rats as compared with 4-month-old rats. Table 3 gives relationships and correlations between plasma osmolality and plasma AVP concentration for different ages and time periods or periods. To obtain more clear relationships for 4-month-old rats, data from three consecutive time points were taken together (morning: 08.00, 10.00 and 12.00, afternoon: 14.00.
16.00 and 18.00, etc.). Relationships for the 24 h period are based on plasma data of rats killed at 08.00, 16.00 and 24.00. The interdependence of plasma osmolality and plasma AVP concentration in 4-month-old rats was affected by the time of day (test for parallelism and coincidence, p-values < 0.001). Time of day did not affect the interrelation in 20- and 31-month-old rats (p-values > 0.05). Age affected the relationship between the osmolality and AVP concentration of plasma collected between 11.00 and 13.00 (test for parallelism, p > 0.05; test for coincidence, p < 0.001), but not the average daily relationship (p-values > 0.05).

### Discussion

In this study we investigated the changes in plasma AVP concentration and osmolality in male Wistar rats in relation to age and time of day.

Plasma AVP concentrations and plasma osmolalities were increased in 21-month-old rats between 11.00 and 13.00 as compared with 3- and 4-month-old rats. The relationship between plasma osmolality and plasma AVP concentration at midday seemed to be affected by age (but see also below). Daily water intake per 100 g body weight diminished with age, but total water intake was unaltered until 21 months of age. Total water intake

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Table 2. Average daily plasma AVP concentration and osmolality in male Wistar rats of different ages. Data represent means ± SEM.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Weight (g)</th>
<th>Plasma osmolality (mOsm/kg)</th>
<th>Plasma AVP concentration (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>335 ± 6</td>
<td>292.6 ± 1.5</td>
<td>1.05 ± 0.40</td>
</tr>
<tr>
<td>20</td>
<td>477 ± 9</td>
<td>300.6 ± 1.1</td>
<td>1.60 ± 0.36</td>
</tr>
<tr>
<td>31</td>
<td>407 ± 17</td>
<td>303.7 ± 1.8</td>
<td>3.44 ± 0.50</td>
</tr>
</tbody>
</table>

Values with different superscripts differ significantly (p < 0.05). Values with identical superscript do not differ significantly.

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Table 3. Relationship between plasma osmolality and plasma AVP concentration at different time points or periods of the day in Wistar rats of different ages.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Time point or period</th>
<th>Relationship</th>
<th>N</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Morning</td>
<td>Y = 31.3 - 0.107X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16</td>
<td>0.812</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>4</td>
<td>Afternoon</td>
<td>Y = 50.7 + 0.178X</td>
<td>12</td>
<td>0.492</td>
<td>ns</td>
</tr>
<tr>
<td>4</td>
<td>Evening</td>
<td>Y = -63.2 + 0.222X</td>
<td>12</td>
<td>0.917</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>Night</td>
<td>Y = -86.6 + 0.302X</td>
<td>12</td>
<td>0.580</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>20</td>
<td>08.00</td>
<td>Y = -165.8 + 0.556X</td>
<td>6</td>
<td>0.533</td>
<td>ns</td>
</tr>
<tr>
<td>20</td>
<td>16.00</td>
<td>Y = -46.9 + 0.163X</td>
<td>7</td>
<td>0.918</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>20</td>
<td>24.00</td>
<td>Y = 3.79 + 0.010X</td>
<td>6</td>
<td>0.455</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>31</td>
<td>08.00</td>
<td>Y = -13.6 + 0.052X</td>
<td>5</td>
<td>0.095</td>
<td>ns</td>
</tr>
<tr>
<td>31</td>
<td>16.00</td>
<td>Y = -89.1 + 0.306X</td>
<td>6</td>
<td>0.204</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>31</td>
<td>24.00</td>
<td>Y = 1.39 + 0.063X</td>
<td>5</td>
<td>0.531</td>
<td>ns</td>
</tr>
<tr>
<td>20</td>
<td>11.00 to 13.00</td>
<td>Y = -27.3 + 0.095X</td>
<td>16</td>
<td>0.664</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>20</td>
<td>11.00 to 13.00</td>
<td>Y = 25.7 + 0.091X</td>
<td>12</td>
<td>0.362</td>
<td>ns</td>
</tr>
<tr>
<td>21</td>
<td>11.00 to 13.00</td>
<td>Y = -15.7 + 0.057X</td>
<td>16</td>
<td>0.346</td>
<td>ns</td>
</tr>
<tr>
<td>21</td>
<td>11.00 to 13.00</td>
<td>Y = -43.7 + 0.154X</td>
<td>16</td>
<td>0.500</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>20</td>
<td>24 h period</td>
<td>Y = -63.2 + 0.220X</td>
<td>12</td>
<td>0.816</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>20</td>
<td>24 h period</td>
<td>Y = -25.7 + 0.091X</td>
<td>19</td>
<td>0.260</td>
<td>ns</td>
</tr>
<tr>
<td>31</td>
<td>24 h period</td>
<td>Y = 59.6 + 0.208X</td>
<td>16</td>
<td>0.751</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

<sup>a</sup>Y = plasma AVP concentration (pmol/l), X = plasma osmolality (mOsm/kg).
was not determined in 31-month-old rats in the present study, but in other studies we did not find alterations in total water intake until 32 months of age (unpublished results). In the literature water intake is usually corrected for body weight. This may not be a reliable procedure, since water intake is probably related to food intake, which has been found not to change in Wistar rats with age (20). Therefore, total water intake is probably a better functional measure of water homeostasis than water intake per 100 g body weight. From the above findings it can be concluded that the regulation of fluid balance is affected in Wistar rats at a relatively early age, although the total water intake remains unaltered.

In the second experiment we observed clearcut daily fluctuations in plasma AVP concentration and osmolality in 4-month-old rats. It is likely that nocturnal rises in plasma osmolality and plasma AVP concentration are related to water- and food intake, and resorption of water and nutrients. However, the observation that time of day influenced the relationship between plasma osmolality and plasma AVP concentration in 4-month-old rats indicates that factors other than osmolality are involved in the generation of the rhythm in plasma AVP concentration. It is possible that the secretion of AVP into the blood is regulated by a circadian oscillator. The most important biological clock in mammals is the suprachiasmatic nucleus (21). Since neurons of this nucleus secrete AVP into the cerebrospinal fluid in a circadian fashion (22–24), it is tempting to speculate that these neurons also secrete AVP into the blood. However, it has not been demonstrated that fibers originating from the suprachiasmatic nucleus terminate abutting blood vessels. Furthermore, the rhythm of AVP concentration in cerebrospinal fluid is opposite to that in blood (22). Therefore the role of the suprachiasmatic nucleus in the generation of the rhythm in plasma AVP concentration is unclear as yet.

Irrespective of absolute values, daily patterns of plasma AVP concentration and plasma osmolality resembled each other in 4- and 31-month-old rats, but were different in 20-month-old rats. This is not surprising if one considers the multiple factors that may influence AVP release with aging, such as circadian changes in kidney function, locomotion and concentrations of neurotransmitters, hormones and receptor densities. Statistical analysis according to the general regression theory did not reveal an effect of time of day on the relationship between plasma osmolality and plasma AVP concentration in 20-month-old rats, although the observation that plasma AVP concentration was related to the time of day and plasma osmolality was not, would suggest otherwise. The influence of time of day on the relationship between plasma AVP concentration and plasma osmolality approached statistical significance in 31-month-old rats (test for coincidence, \( p = 0.072 \)).

The relationship between plasma osmolality and plasma AVP concentration between 11.00 and 13.00 seemed to be age-related, but the average daily relationship did not. This apparent inconsistency probably reflects the change in the circadian pattern of plasma AVP concentration with age. It can be concluded that osmoreceptor sensitivity does not change in Wistar rats with aging.

In contrast to the plasma AVP concentration during the light period the average daily AVP concentration was increased in 31-month-old Wistar rats only. This is in accordance with the results of Fliers and Swaab (3), who obtained morphometrical evidence for an increased neurosecretory activity in the paraventricular nuclei of 32-month-old Wistar rats.

Although the present findings are consistent with the findings of Fliers and Swaab (3), they are not consistent with those of others (1, 2, 8, 25, 26). Factors that may have contributed to the conflicting data on the fluid balance in aging studies are increased handling stress in old rats (8), a seasonal rhythm in plasma AVP concentration (2, 27) and strain differences. With respect to our findings the influence of stress can be excluded. First, we handled our rats prior to experimentation. Second, plasma osmolality and plasma AVP concentration showed a high correlation in aged rats. This would not have been the case if stress was the cause of the increased plasma AVP concentrations. It can also be excluded that our data are affected by a seasonal rhythm in plasma AVP concentration, since irrespective of the season we always found plasma AVP concentrations of about 0.5 pmol/l in Wistar rats (measured in Nov, Feb, June and Aug in 9 to 16 animals of 3 to 4 months of age, \( p > 0.05 \), ns, unpublished results). The influence of age on fluid balance seems to vary less within strains than between strains. So it seems reasonable to assume that there are real strain differences in this respect.

The present findings with respect to the altered fluid balance in aged Wistar rats, in general, resemble findings made in aged human subjects. Reduced kidney function is generally found in humans. Although we did not measure kidney function, the present findings suggest a reduced sensitivity of the kidney to AVP in aged Wistar rats. Most studies also report increased plasma AVP concentrations in human subjects (28–33), but not increased plasma osmolalities (28, 33). However, plasma osmolalities reported in the literature may not be reliable, because frozen plasma samples were used, and freezing increases the intra-assay coefficient of variation (17). We found a gradual increase in osmolality in healthy human volunteers aged 17 to 60 years (unpublished results) when we used a protocol quite similar to that of Os et al. (33), who did not find an increase in osmolality. Taken together, it can be concluded that Wistar rats may provide an appropriate model for the study of the influence of aging on fluid balance in humans.

In conclusion, the present findings suggest that plasma AVP concentration and plasma osmolality increase with age in male Wistar rats, and that the daily
pattern of plasma AVP concentration and plasma osmolality change during the aging process. We did not find a changed relationship between plasma AVP concentration and osmolality with age, lending support to the notion that the primary disturbance in water homeostasis is a kidney deficit as opposed to an increased release of AVP into the blood. The total water intake appeared to be constant in Wistar rats during aging, so that a decreased kidney function is offset by a higher osmolality and a subsequent rise in plasma AVP concentration.

References


