Elevated serum oxytocin of the vasopressin-deficient Brattleboro rat is present throughout life and is not sensitive to treatment with vasopressin

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Abstract. The postnatal developmental course of the enhanced OT serum level of the vasopressin-deficient (homozygous) Brattleboro rat was investigated radioimmunologically together with the response to treatment with Pitressin tannate. Compared with heterozygous Brattleboro (control) pups, in which serum OT appeared to have an adult value from birth onwards (about 10 pmol/l), homozygous rats had approximately 2-fold enhanced OT serum levels throughout early development. Between day 55 and adulthood the levels of OT rose further to 40–50 pmol/l. A 3-day treatment with Pitressin tannate both in the period before or after the age (day 16) at which the polyuria of the homozygous Brattleboro mutant can be revealed, failed to reduce the serum OT. It was therefore concluded that the high OT serum levels in the vasopressin-deficient Brattleboro rat are not induced by osmotic imbalance, but probably originates from functional teratological aspects of the mutation.

Owing to a disturbed synthesis of the antidiuretic hormone vasopressin in the hypothalamus, the homozygous (HOM) Brattleboro mutant rat revealed a severe diabetes insipidus (Valtin 1982). This chronic osmotic stress is thought to activate oxytocin-synthesizing neurons in the same nuclei of the hypothalamo-neurohypophyseal neurosecretory system (cf. Morris 1982) resulting in decreased OT levels in the neural lobe of the pituitary (Valtin et al. 1965) and in enhanced serum OT (Balment et al. 1982; North et al. 1982). Though high OT levels have an antidiuretic effect (cf. Sawyer & Valtin 1967), the magnitude of elevated serum levels of OT in the HOM Brattleboro rat is probably insufficient to cope with the impaired antidiuresis at the kidney level (Edwards et al. 1982). Compensatory responses of OT neurons have also been proposed for the absence of central actions of AVP (Baertschi & Bény 1982), but reports on e.g. elevated OT levels in the cerebrospinal fluid of the HOM rat are not at hand.

Increased OT activity is claimed to alter or impair several physiological processes in HOM Brattleboro rat (Boer et al. 1982a; Haldar et al. 1982; Hanif et al. 1983), many of which could only partially be restored by AVP supplementation. It was therefore assumed that other defects were present, which may even have been induced by enhanced OT levels early in development (cf. Goren et al. 1982). However, it is not known whether high levels are indeed present perinatally. Nevertheless, there are some indications for an activated OT system in the early development of the HOM Brattleboro rat (when compared with heterozygotes (HET) or Long-Evans control): a) the OT-associated neurophysin can be immuno-visualized one day earlier in prenatal life (Wong et al. 1982); b) radioimmunoassay of fetal pituitary OT already showed reduced levels on postcoital day 20 (Oosterbaan et al. 1985), and c) OT mRNA assays of the hypothalamus demonstrated an advanced gene expression at fetal day 20 (as well as higher levels throughout the developmental period (Van Tol 1987; Van Tol et al., submitted)).
It was surprising to find out that hardly any report has been published on serum OT levels in response to AVP supplementation of HOM Brattleboro rats. Only in a single study by Lutz-Bucher & Koch (1982) has the effect of a 3-week AVP therapy on the serum OT of the adult HOM rat been mentioned. No serum OT response was observed, but this phenomenon was not discussed by the authors.

The present study provides the lacking information on serum OT levels in postnatal development of the Brattleboro strain. The response to short-term AVP supplementation both in neonates and adults had additionally been investigated radioimmunochemically in order to see whether the OT levels are sensitive to a reduced polyuria of these mutants.

**Material and Methods**

**Animals**

Male and female HOM Brattleboro (di/di) and outbred normal (+/+) male rats, weighing 175–200 g, were obtained from CPB/TNO (Zeist, The Netherlands). All animals were kept in large metal cages, and received tap water and standard chow ad libitum. Siring of the HOM females by either HOM or outbred normal males was used to obtain homogenously genotyped litters of the HOM (AVP-deficient homozygotes) or HET trait (heterozygote di/+ controls). This breeding was set up following the strict schedule as described previously in order to eliminate any influence (environmental or maternal) on the outcomes except for those related to the mutation, i.e. virtual absence of AVP (Boer et al. 1982b). The day of birth was called day 0 postnatally.

**Oxytocin assay**

For the assay of serum OT, rats were decapitated between 10.00 and 12.00 h, and trunk blood was collected in chilled plastic tubes. After centrifugation at 3000 x g for 20 min at 4°C, the serum was decanted into another plastic tube and stored frozen at −25°C until assay.

Radioimmunoassay was performed after Vycor glass extraction of the 0.5 to 1.5 ml samples according to the procedures of Dogterom et al. (1977). The duplicate two-dilution samples of the sera extracts showed parallelism with the standard OT curve. Detection limit of the assay was 3 pmol/l serum.

**Developmental series**

Whole litters were sacrificed on postnatal day 1, 8, 12, 16, 21, 24 and 55. Additional groups of day-55 (see Results) as well as adult (about 6 months) HOM and HET rats, directly obtained from the animal supplier, were sacrificed after a week of adaptation in our own animal facilities. For pups younger than 24 days, blood was always pooled, whereas for day-55 and adult animals blood was collected individually. Whenever it was possible in the young ages to collect enough serum for an assay (≥ 0.5 ml), male and female serum from one litter was pooled separately. For the 1- and 8-day-old pups, it was necessary to pool serum from 2 or more litters. The number of pups used to get single samples for the assay ranged between 12 to 28 pups for day 1, between 8 to 16 for day 8, between 5 to 9 pups for day 12 and 16, and between 3 to 7 pups for day 21 and 24 of age. Small aliquots of serum (100 µl) were used to measure osmolality.

**Pitressin tannate treatment**

In three separate series, HOM Brattleboro rats were injected between 10.00 and 11.00 h for 3 subsequent days with Pitressin® tannate (5 kU/l; Parke-Davis), a long-acting AVP preparation in oil. Pups up to the age of 10 days received a sc injection of 0.25 U daily, older pups 0.5 U daily, whereas day-55 and adult rats got 5 U/kg per day (cf. Wright & Kutscher 1977). Volumes of arachis oil (Brocades) were used as control treatment. In water extracts of Pitressin tannate, radioimmunoassayable OT was below 5% of the value for AVP (data not presented).

In the first series, HOM pups of postnatal day 0, 8 and 12 were postnatally randomly divided into oil and Pitressin tannate groups, and serum was collected 4 days later as described above. The number of litters used was 18, 7 and 5, respectively. In the second series, each of the 10 HOM litters of either 4- or 12-day-old pups was divided into 3 groups. One group received the handling of the injection procedure (reference), the other two groups were the oil control and Pitressin tannate-treated pups. In the third series one group of 14 adult HOM males was individually housed in metabolism cages and divided and treated similarly as in the first series, whereas another group of 6 males were treated first with oil for 3 days and then for 3 days with Pitressin tannate. Body water metabolism was monitored and urine osmolality determined in both groups. In the latter, a 100–200 µl blood sample was daily withdrawn from the tail vein for the test of serum osmolality throughout the injection period.

**Statistics**

Two-way variance analysis for unequal group sizes was performed on the developmental data (Winer 1971), whereas for a direct comparison of the group data Studentized Range statistics were applied (Winer 1971). Statistical evaluation of the Pitressin tannate treatment was assessed similarly. Significance of difference was taken at $P < 0.05$. 

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Fig. 1.
Serum oxytocin levels of developing Brattleboro rats. Individual data of pooled samples of heterozygote control (o) and homozygote pups (●) are given together with the median values for each age (bars). Analysis of variance showed no age-related change (MS\text{within cell} = 78, F_{5,61} = 2.2), but significantly different values between genotypes for the whole postnatal period (F_{1,6} = 17) as well as for all ages (asterisks), with postnatal day-24 as the exception (▲ or ▼ denote assay result above upper or lower level of detection, see also Figs. 2–4).

Fig. 2.
Median serum oxytocin levels of 55-day-old and adult Brattleboro rats, being either heterozygote (control, o) or homozygote (●) for the mutation. Analysis of variance showed an effect of age for the females (F_{1,52} = 37), not for the males (F_{1,47} = 1.1), whereas an effect of genotype (asterisks) was apparent for both sexes (F_{1,52} = 26 and F_{1,47} = 15, respectively), though for day-55 males direct comparison of group data failed to reach statistical significance. A sex difference was only seen for plasma levels of heterozygous adults, the females having higher levels than the males.
Results

**Oxytocin levels in developing heterozygous Brattleboro rats**

For the Brattleboro HET pups, OT serum levels remained at a level of about 12 pmol/l throughout the first postnatal month (Fig. 1). Thereafter levels were not much different (Fig. 2), except for a 2-fold increase for HET females between day-55 postnatally (8.0 pmol/l) and adulthood (16.6 pmol/l; 0.025 < P < 0.05). Statistical evaluation of the neonatal data of Fig. 1 (referring to both male or female pooled plasma) together with the data of older animals, given separately for both sexes in Fig. 2 showed that a sexual dimorphism was only present in adulthood: female level was then higher (16.6 vs 6.1 pmol/l, 0.01 < P < 0.05; Fig. 2).

**Comparison with the homozygous Brattleboro rat**

Serum hyperosmolality of the AVP-deficient HOM Brattleboro rats becomes visible at day 21 (Table 1). Up to this age, serum OT levels of the HOM pups were about 2-fold higher than those of the HET controls (P < 0.001; Fig. 1). On day 24, and on day 55 for the males, no statistically significant difference (P > 0.25) was, however, found between the genotypes, whereas for day-55 females as well as for adults of both sexes an enhanced serum OT was again demonstrable (Fig. 2). The most obvious increases were seen in adults, the levels of which were raised to 40–50 pmol/l. Since the latter result was obtained with animals directly obtained from the animal supplier (raised from HET mothers) and the much lower increase at day-55 was seen for animals from our own animal house (breeding with HOM mothers), additional groups of day-55 bred by the supplier were investigated. However, the different developmental history caused no change in OT serum level, for either genotype (data not presented), and data were therefore pooled.

**Vasopressin treatment**

The 3-day Pitressin tannate treatment reduced the polyuria of adult HOM Brattleboro rats, but failed to decrease the serum hyperosmolality statistically significant (Table 2). Since the variability of such serum data among individual rats has been reported to be considerable (see Discussion), serum osmolality was determined in daily samples of tail vein blood before and after the treatment in another group (Table 3). Both polyuria and serum hyperosmolality then showed a significant decrease upon treatment with the AVP preparation.

In the two separate developmental series, Pitressin tannate injections of HOM Brattleboro rats to

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**Table 1.** Plasma osmolality of developing Brattleboro rats.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Heterozygotes (controls) (mosmol/kg H₂O)</th>
<th>Homozygotes (mosmol/kg H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>309 ± 6 (5)</td>
<td>304 ± 5 (5)</td>
</tr>
<tr>
<td>12</td>
<td>291 ± 8 (8)</td>
<td>307 ± 11 (8)</td>
</tr>
<tr>
<td>21</td>
<td>283 ± 4 (4)</td>
<td>300 ± 3 (4)*</td>
</tr>
<tr>
<td>24</td>
<td>284 ± 3 (6)</td>
<td>297 ± 4 (8)</td>
</tr>
<tr>
<td>55</td>
<td>287 ± 2 (10)</td>
<td>307 ± 7 (8)*</td>
</tr>
<tr>
<td>Adult</td>
<td>288 ± 4 (10)</td>
<td>304 ± 3 (10)*</td>
</tr>
</tbody>
</table>

Data are average ± SEM (N). Analysis of variance resulted in significance of difference between the genotypes (F<sub>1,74</sub> = 5.1, P < 0.05).

* Statistically significant difference for a particular age.

**Table 2.** Osmotic parameters of male homozygous Brattleboro rats after a 3-day treatment with vasopressin (Pitressin tannate).

<table>
<thead>
<tr>
<th></th>
<th>Untreated/ oil</th>
<th>Pitressin tannate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>347 ± 23</td>
<td>387 ± 28</td>
</tr>
<tr>
<td>Urine production (ml/kg per day)</td>
<td>552 ± 28</td>
<td>31 ± 8*</td>
</tr>
<tr>
<td>Urine osmolality (mosmol/kg H₂O)</td>
<td>154 ± 17</td>
<td>1310 ± 150*</td>
</tr>
<tr>
<td>Plasma osmolality (mosmol/kg H₂O)</td>
<td>290 ± 2</td>
<td>295 ± 3</td>
</tr>
</tbody>
</table>

Daily dosage was 0.5 U Pitressin tannate in 0.1 ml oil per 100 g body weight, with similar volumes of oil as control. Untreated and oil-treated control data were pooled since no difference was found between these groups (cf. adults of Fig. 4). Data are given as average ± SEM.

* Statistically significant difference at P < 0.05 (Student's t-test).
Table 3.
Course of osmotic parameters of male homozygous Brattleboro rats before and after daily treatment with Pitressin tannate.

<table>
<thead>
<tr>
<th></th>
<th>Control period (days)</th>
<th>Pitressin tannate period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>288 ± 11</td>
<td>289 ± 10</td>
</tr>
<tr>
<td>Urine production (ml/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>843 ± 51</td>
<td>859 ± 47</td>
</tr>
<tr>
<td>Urine osmolality (mosmol/kg H₂O)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>157 ± 6</td>
<td>167 ± 5</td>
</tr>
<tr>
<td>Serum osmolality (mosmol/kg H₂O)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>309 ± 13</td>
<td>330 ± 7</td>
</tr>
</tbody>
</table>

Daily dosage of Pitressin tannate, see legend to Table 2. Data are averages ± SEM for 6 rats.
* Statistical significant difference with the control period.


treat the AVP-deficiency (all ages) and to reduce the polyuria (from day 16 onwards, see Discussion) failed to reduce the enhanced serum OT at any of the postnatal ages tested or in adulthood (Figs. 3 and 4).

Discussion

Neonatal course of serum oxytocin in controls
A few other studies report on serum levels of OT in the developing rat (Wolf et al. 1984; Higuchi et al. 1985; Carter & Lightman 1986). The present data on developing HET (AVP-synthesizing) Brattleboro rats largely revealed the same pattern as seen in other strains: a) a sex difference in plasma OT is absent (cf. Carter & Lightman 1986), and b) absolute data are comparable to those of Carter & Lightman (1986), but slightly below the postnatal level of approximately 19 pmol/l reported by Higuchi et al. (1985). The major discrepancy appeared to be the absence of low OT levels during the first few days after birth: for HET Brattleboro rats serum OT immediately after birth was of a value found at the later stages of life. This may point to an enhanced maturation of the OT release in these rats, since for the other strains immunocytochemical and radioimmunoassay data of OT in the rat hypothalmo-neurohypophyseal system at least confirm the generation of significant amounts of OT after, but not before birth (cf. review by Boer 1987).

The influence of the hereditary vasopressin deficiency
The well-described enhanced OT serum level of the AVP-deficient HOM Brattleboro rat (see introduction) has been confirmed both in males and in females and appears to be present throughout life. The 3- to 5-fold increase for the adults, however, exceeded the approximately 2-fold

Fig. 3.
Absence of influence of a 3-day vasopressin treatment on the serum oxytocin levels of homozygous Brattleboro pups. Assay points of pooled samples are given together with the median values for oil-treated control (open bars) and Pitressin tannate-treated pups (hatched bars). Analysis of variance revealed no influence of the treatment (F_{1,31} = 0.003).
higher levels seen throughout the postnatal period up to day 55 of age (possible with a dip around day 24, cf. Fig. 1). Thus, increased release of OT is present a) shortly after rat's delivery and may have its onset even before birth, and appeared b) to be amplified after day 55. Recent findings of enhanced OT mRNA levels in the brain of the HOM rat between fetal day 20 and postnatal day 30 (Van Tol 1987; Van Tol et al., submitted) are fully in agreement with an early activation of OT synthesis. However, during the first two postnatal weeks it is unlikely that it is induced by osmotic stress of the HOM pups. The syndrome of diabetes insipidus is absent before 16 days of age (Dlouhá et al. 1982) and serum hyperosmolality develops after this age (Table 1), whereas kidney of normal rat becomes responsive to AVP only around postnatal day 8–10 (Rajerison et al. 1976).

AVP supplementation was performed both in neonatal and in adult HOM rats in order to investigate whether serum OT indeed was not subject to osmoregulation in this mutant and whether there possibly was a developmental change in response around day 16. Pitressin tannate rather than pure AVP was chosen for treatment because of the long-acting character of the former. Moreover, a 3-day treatment was preferred to a longer treatment, since only then the effects of 3-day-olds and older pups could be compared, and since neurohypophyseal hormone levels are known to respond within a day of altered osmotic demands (cf. Kruisbrink et al. 1988). Daily Pitressin tannate injections to adult HOM rats for 3 days suppressed the syndrome of diabetes insipidus, but failed to change the serum OT. The observation that serum osmolality did not respond statistically significant in one group (Table 2) confirms a study by Bouby et al. (1984) with the non-pressor antidiuretic AVP agonist 1-deamino[Arg8]VP (dDAVP) and appeared to be due to variability among animals. When the animals serve as their own controls, a clear-cut drop of serum osmolality can be measured within 24 h (Table 3). Thus, the 3-day Pitressin tannate treatment (5 U/kg per day) takes away the osmotic stress of the adult HOM Brattleboro rat, but serum OT remained enhanced. This is in full agreement with the single figure given by Lutz-Bucher & Koch (1982). The increase of pituitary OT content towards control levels upon long-term treatment with AVP (cf. Balment et al. 1982)
may, therefore, not necessarily be interpreted as a decrease of release from the neurosecretory terminals. Long-term treatment of adult HOM Brattleboro rats with AVP (Rehbein et al. 1986) or dDAVP (Burbach et al. 1986) additionally failed to decrease the enhanced OT gene expression showing that the synthesis of the OT precursor is not affected. Therefore, the enhanced OT activity in the HOM Brattleboro rat do not seem to be controlled by plasma osmolality or by peripheral AVP. The absence of osmo-imbalance of the HOM pups before 2 weeks of age (Table 1; Dlouhá et al. 1982; Babicky et al. 1986), supports the notion. AVP is apparently superfluous for osmotic control mechanisms in this early stage, nevertheless serum OT is enhanced and again insensitive to AVP treatment. Thus, other factors are being responsible for the alterations and the intriguing question of what induces the stimulated OT release therefore remains.

Developmental aspects

It might be assumed that the early developmental absence of AVP has changed the organization of the OT system in a permanent way. Cross-breeding of the Brattleboro mutation into another rat strain has indeed shown that a reduced neural lobe OT content is again coupled to the AVP deficiency (Ganten et al. 1983). The augmented gene expression of hypothalamic OT mRNA, but also the delayed expression of the mutant AVP mRNA in the HOM fetus (Van Tol et al. 1986, Van Tol 1987) might additionally be indicative of altered maturation owing to the lack of AVP production. Such early imbalances may have caused a different 'setting' of the basal activity of the neurons (cf. Boer & Swaab 1985; Csaba 1986). The early activation of OT cells in ontogenesis, however, needs not necessarily be restricted to the sites of synthesis in the brain. The adrenal of the HOM Brattleboro rat also synthesizes OT (as well as AVP, Nussey et al. 1984), whereas the fetal adrenal contains OT (Ravid et al. 1986) and neither fetal brain nor maternal circulation contributes to amniotic fluid OT levels in the rat (Swaab & Oosterbaan 1985). However, a possible peripheral source of circulating OT is still speculative and requires early adrenalectomy experiments.

Neonatally enhanced AVP induces a diabetes insipidus in later life (Wright & Kutscher 1977; Handelmann et al. 1983; Boer et al. 1984) by permanent down-regulation of kidney AVP receptors (Handelmann & Sayson 1984). This is also seen with congeners of AVP, including OT (Snijdewint & Boer 1986). Similarly early exposure to endogenously enhanced OT might have affected the OT receptor setting in a permanent way. Lower sensitivity for OT has in fact been reported for uterus contraction (Haldar et al. 1982) and for lipolysis in adipocytes of the HOM rats (Hanif et al. 1983), defects that, moreover, could not be completely normalized by AVP treatment. In turn, the decreased magnitude of post-receptor signals may increase the demands for OT, i.e., they potentiate the high OT serum level. This ongoing process may have induced the further increase in serum OT observed between 55-day-old and adult HOM Brattleboro rats, a phenomenon also seen in the normal aging rat (Fliers & Swaab 1983).

HOM Brattleboro rats have an impaired body and brain growth (cf. Boer 1985). To substantiate a possible role for the lack of AVP in this phenomenon, perinatal AVP supplementation studies were performed to improve growth, but with only little success (Boer 1985; Snijdewint et al. 1985). The present results may imply that such treatments will fail to reduce the enhanced OT levels. Since both enhanced maternal OT serum levels (Boer & Kruisbrink 1984) and postnatal treatment with OT of normal Wistar pups (Snijdewint & Boer 1986) revealed (temporary) growth disturbances, the influence of OT in the disturbed growth of the HOM rats should be taken into account as well.

It is not clear why the fetal absence of AVP induces the permanent up-regulation of OT synthesis. It could be that normally AVP modulates the maturation of the OT system. A woman with a central diabetes insipidus, i.e. nondetectable serum AVP, but onset of the syndrome at later age, failed to show enhanced OT serum levels (Shangold et al. 1983). This observation reinforces the the possibility that only the absence of AVP during the period of early brain development may have a lasting effect on the OT system. The above findings may be indicative of possible adverse effects of imbalances of neuropeptides during nervous system development. The functional teratological aspects of these compounds still need further investigations, since their clinical applications are becoming popular (Boer & Swaab 1985; Kastin et al. 1987).
Acknowledgments

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