Vasopressin gene expression is attenuated in the fetal Brattleboro rat

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Due to a genetic defect the homozygous Brattleboro rat is unable to synthesize vasopressin gene products but still transcribes a mutant vasopressin mRNA from the gene. To study the influence of vasopressin gene products on the development of vasopressin gene expression, vasopressin mRNA levels of the supraoptic and paraventricular nucleus were measured at fetal day 20, postnatal day 1, 15 and 30 in the Wistar rat and in the heterozygous and homozygous Brattleboro rat by Northern blot analysis and in situ hybridization. In the homozygous Brattleboro rat of fetal day 20 and postnatal day 1, no or minute amounts of vasopressin mRNA were detectable but vasopressin mRNA was readily detectable at postnatal day 15 and 30. The Wistar rat and heterozygous Brattleboro rat had abundant vasopressin mRNA at fetal day 20 with increasing amounts towards postnatal day 30. The results indicate that vasopressin gene expression in the development of the homozygous Brattleboro rat is attenuated, possibly due to the absence of vasopressin gene products.

Vasopressin *Gene expression* *Development* *(Brattleboro rat)*

1. INTRODUCTION

The homozygous (di/di) Brattleboro rat has no pituitary vasopressin (VP) and consequently suffers from severe diabetes insipidus [1]. The failure of hypothalamic nuclei to synthesize VP has been proposed to be due to the one base deletion in the VP gene [2]. The mutant VP gene is transcribed and spliced correctly, but the mutant mRNA is inefficiently translated [3,4]. There is transcription from the mutant VP gene, but it is unknown whether this gene is normally regulated. In normal animals VP gene expression is strongly enhanced during the first month after parturition as indicated by the VP mRNA content of the hypothalamic nuclei, i.e. the supraoptic nucleus and paraventricular nucleus [5]. Here we report that before birth these nuclei of the homozygous (di/di) Brattleboro rat are devoid of detectable VP mRNA, in contrast to those of the heterozygous (+/di) Brattleboro and Wistar rats. The data show that the expression of the VP gene is attenuated in the fetal and neonatal di/di rat, but is subject to upregulation during postnatal development. It is suggested that this defect may be related to the absence of VP gene products during the development of the fetal Brattleboro rat.

2. MATERIALS AND METHODS

2.1. *Animals*

For Northern blot analysis male Wistar rats, heterozygous (+/di) Brattleboro rats and homozygous (di/di) Brattleboro rats at the age of fetal day 20 (f20), postnatal day 1 (p1), 15 (p15) and 30 (p30) were decapitated. Brain sections containing the supraoptic nucleus (SON) and paraventricular nucleus (PVN) were isolated from the hypothalamus by microdissection [6] and stored at −80°C.

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For in situ hybridization brain tissue of male Wistar rats and homozygous (di/di) Brattleboro rats at the age of postnatal day 1 (p1) and 30 (p30) was fixed by perfusion with 4% formaldehyde in phosphate buffered saline (PBS), left overnight in 15% sucrose in PBS and stored at −70°C.

To exclude maternal effects the offspring of both Brattleboro breedings originated from female di/di rats sired by di/di and +/di males, respectively [7].

2.2. *Northern blot analysis*

From each experimental group consisting of 4 animals, total RNA was isolated and analyzed on Northern blots as described [8]. The relative levels of VP mRNA on the Northern blots were quantitated by determination of the absorbance of the autoradiographic signals with a video image analysis, processing, evaluating and recording system (VIPER, Gesotec, Darmstadt). Auto radiographics of dot blots with known amounts of M13 DNA containing exon C, hybridized with the VP-specific ^{32}P-labeled probe, were used for standardization of the absorbance.

2.3. *In situ hybridization*

Hybridization on brain slices with single-stranded ^{35}S-labeled VP-specific DNA probe was in 50% formamide and 10% dextran sulphate on 16 μm cryostat sections for 18 h at 37°C following a modified procedure of Lawrence and Singer [11,12]. After hybridization sections were rinsed in 50% formamide, 2 × SSC at 37°C for 30 min, 50% formamide, 1 × SSC at 37°C for 30 min, and 1 × SSC at room temperature for 30 min. Slides were dipped in ethanol, air-dried and exposed to Fuji RX X-ray film for 7 days. The VP-specific probe was a single-stranded ^{35}S-labeled genomic DNA fragment of exon C of the rat VP gene [10]. The probe (314 bases) was enzymatically synthe-

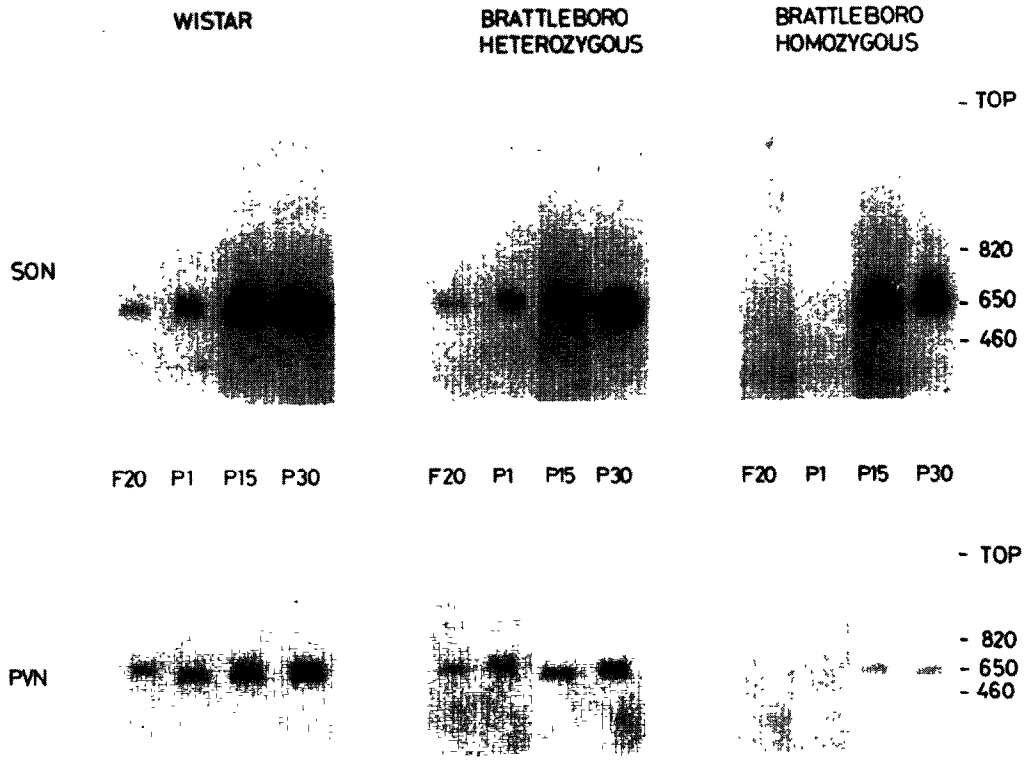

Fig.1. Analysis of VP gene expression in SON and PVN of male homozygous (di/di), heterozygous (+/di) Brattleboro rats and Wistar rats of fetal day 20 (f20), postnatal day 1 (p1), 15 (p15) and 30 (p30). The autoradiograms of the Northern blots show hybridization of VP mRNA in SON (upper) and PVN (lower).

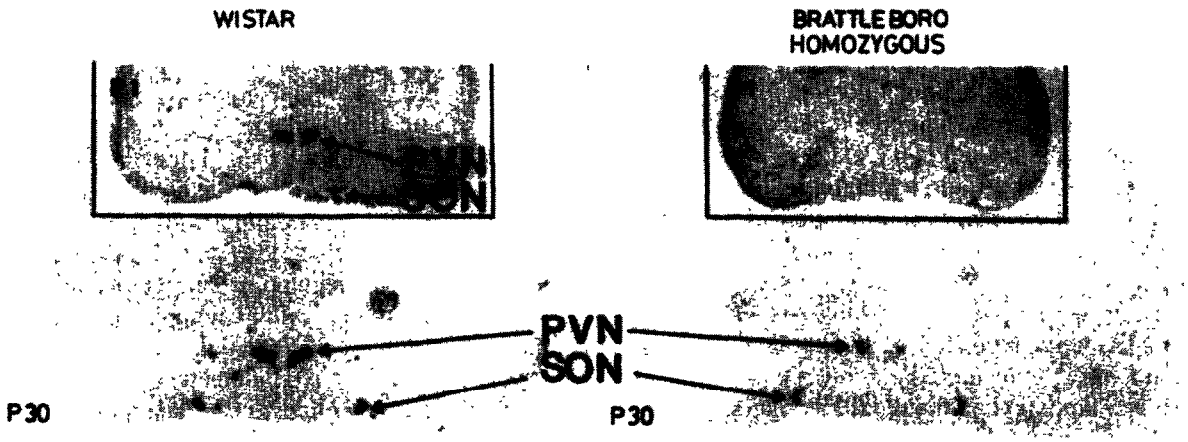

Fig.2. In situ hybridization of VP mRNA in hypothalamic brain slices of male homozygous (di/di) Brattleboro rats and Wistar rats at the age of postnatal day 1 (p1) and 30 (p30). SON, supraoptic nucleus; PVN, paraventricular nucleus.

sized by primer extension in the presence of ^{35}S-labeled deoxycytidine 5′-triphosphate using a recombinant M13 clone (MPB-2) as template [8] and obtained after restriction digestion and polyacrylamide-urea gel electrophoresis as single-stranded ^{35}S-labeled DNA.

3. RESULTS

VP mRNA contents of microdissected SON and PVN of male homozygous (di/di), heterozygous (+/di) Brattleboro rats and Wistar rats were analyzed at the age of fetal day 20 (f20), postnatal day 1 (p1), 15 (p15) and 30 (p30). Total RNA was isolated from SON and PVN and analyzed on Northern blots in two independent experiments. The Northern blots were hybridized with a VP mRNA-specific ^{32}P-labeled DNA probe [8] and revealed that the +/di and Wistar rats contained readily detectable amounts of VP mRNA in both SON and PVN at all ages. In contrast, the di/di rats had no detectable amounts of VP mRNA at f20 in the two hypothalamic nuclei. VP mRNA was not detectable in the PVN of di/di rats at p1 as well, but in one of the two experiments a very faint band corresponding to VP mRNA was observed in the SON of di/di rats at p1 (fig.1). At p15 and p30 the VP mRNA content of the SON and PVN of the di/di rats had increased as in +/di and Wistar rats. Examination of the autoradiograms of the Northern blots by computerized image analysis showed that the PVN of the di/di rats had no detectable VP mRNA at f20 and p1 and in the SON at f20. At p1 the di/di rats had at least 20-fold lower VP mRNA levels in the SON than +/di and Wistar rats. At p30 the VP mRNA levels of SON and PVN of di/di rats were approx. 66% of those of the +/di and Wistar rats.

In situ hybridization was used to evaluate anatomically the above observations. For this purpose brains of di/di and Wistar rats of p1 and p30 were used. In situ hybridization of brain slices was performed with a VP mRNA-specific single-stranded ^{35}S-labeled DNA probe. At p1 the di/di rat did not contain detectable amounts of VP mRNA, while at p30 VP mRNA was detected in the SON and PVN (fig.2). In Wistar rats VP mRNA was readily detected at p1 and p30.

4. DISCUSSION

The presence of readily detectable amounts of VP mRNA in the SON and PVN of Wistar and heterozygous (+/di) Brattleboro rats at f20 demonstrates that the VP gene is expressed prenatally. This observation is in agreement with the immunohistochemical staining of VP- and neurophysin-like material in rat hypothalami as early as f16 [13,14] and the detection by radioimmunoassays of VP and neurophysin immunoreactivity around f14 [15,16]. It indicates that peptides encoded by the VP gene are actually synthesized

prenatally. In the homozygous (di/di) Brattleboro rat, which fails to synthesize VP, its associated neurophysin and glycopeptide [10], the prenatal and early postnatal expression of the mutant VP gene differs markedly, since the mutant VP mRNA is undetectable around birth. This finding is not likely to be attributed to maternal influences, either the absence of VP gene products or the chronically elevated plasma osmolality of the mother, since di/di and +/di rats used in the experiments were bred in di/di mothers. Therefore the attenuated expression of the mutant VP gene around birth is an intrinsic property of the fetal di/di rat. Apparently, the di/di fetus suffers from a defect in the expression of the VP gene either at the level of VP gene transcription or VP mRNA stability. It may be speculated that the permanent absence of VP gene products during the prenatal stage of development has suppressed or prevented the early expression of the mutant VP gene in the di/di Brattleboro fetus. The present results do not allow us to conclude whether this is a direct or a indirect consequence of the absence of VP gene products.

The expression of the mutant VP gene of the di/di rat is nevertheless subject to enhancement in the first postnatal month, as also seen in Wistar [5] and +/di controls, indicating that the mutant gene is sensitive to regulatory factors. This is demonstrated by the VP mRNA contents of SON and PVN in di/di rats at p15. In normal animals this enhancement matches the maturation of the VP neurons at the level of synaptic input and dendritic outgrowth [17,18] and coordinates with the onset of kidney function and the involvement of VP in the control of diuresis [19]. In di/di Brattleboro rats the increae in VP mRNA levels coincide with the manifestation of the diabetes insipidus syndrome, i.e. polydipsia and polyuria, which appears at the onset of the weaning period [19]. It could be speculated that the expression of the mutant VP gene is stimulated for adaptation to impaired osmoregulation. However, at more mature stages, i.e. p30, the VP mRNA levels in the di/di rat appeared to be even lower than those in the +/di and Wistar rats. Previous studies in di/di Brattleboro rats on the basal levels of the mutant VP mRNA and the effects of hyperosmolality have been contradictory [4,20–24]. In our experiments the reported elevated plasma osmolality of 30-day-old di/di rats [19] did not lead to elevated levels of the mutant VP mRNA as compared to +/di and Wistar controls. Still, the neurosecretory neurons of the hypothalamo-neurohypophyseal system of the di/di Brattleboro rat respond to hyperosmolality as indicated by the cellular morphology of VP neurons [25] and the elevated levels of OT mRNA in the di/di rat (unpublished). Therefore, the di/di Brattleboro rat may have another setting of the mechanisms regulating the expression of the VP gene, e.g. another rate of synthesis or degradation of the mutant VP mRNA. Alternatively, it cannot be excluded that defects other than the single base deletion underlie the attenuated expression of the mutant VP gene. Further experiments changing the demand of VP, such as possibly VP supplementation protocols, may help to unravel this question.

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