Vasopressin Gene Expression in Pregnant Rats

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INTRODUCTION

Primary peripheral arterial vasodilation with relative underfilling of the arterial circulation occurs in early pregnancy and leads to several consequences, including decreased systolic and diastolic blood pressure, enhanced cardiac output secondary to afterload reduction, stimulation of renin-angiotensin-aldosterone axis, nonosmotic stimulation of thirst and vasopressin release, and renal sodium and water retention with expansion of the extracellular fluid and plasma volume compartment[1]. The decline in body tonicity during normal human pregnancy starts with conception and is statistically significant during the fifth gestational week, reaches a nadir 10 mosm/kg lower than non-pregnant levels by week 10, after which the decline is sustained until term[2,3]. In Sprague-Dawley rats, the process is slower; significant decrements in plasma osmolality (Posm) can be seen from day 10, and the nadir occurs 4 days later (term=21 days)[4]. This hypoosmolality is apparently due to sustained nonosmotic stimulation of arginine vasopressin (AVP) release from the pituitary gland, even in the state of 30~50% increase in extracellular volume. However, it is not known yet whether the sustained AVP release is also associated with hypothalamic AVP biosynthesis. The present study was therefore undertaken to study the AVP gene expression in the hypothalamus of pregnant rats.

METHODS AND MATERIALS

Rat AVP and oxytocin (OT) cDNA probes were provided by Drs. H. Schmale and D. Richter (Hamburg, Germany). Rat AVP antibody (#2849) for AVP radioimmunoassay was produced by Dr. Jacques Durr (Denver, CO, USA). In vitro transcription kits and restriction enzymes were obtained from Promega (Madison, WI), ^{35}S-UTP was obtained from Amersham (Arlington Heights, IL), and all other chemicals were molecular biology grade.

Timed pregnant Sprague-Dawley rats were purchased along with age-matched virgin rats from a commercial vendor (Harlan, MI). On 20th day (1 day prior to labor) of pregnancy, all the rats of both groups were killed by decapitation, the hypothalamus and pituitary were then rapidly dissected, quickly frozen in the dry ice, and stored at −80 ℃ until extraction of total RNA as described in previous studies[5~7]. Trunk blood was collected for plasma AVP and other electrolytes.

1. Extraction of Total RNA

Total RNA of the rat hypothalamus was extracted by a modified method of Chomzynski and Sacchi[8]. Briefly, the frozen hypothalamus was homogenized in 500 μl of homogenizing solution (4 M guanidinium thiocyanate, 25 mM Na citrate, pH 7.0, 0.5% scarcosyl and 0.1 M 2-mercaptoethanol) by sonication with a microtip. Then 50 μl of 2 M sodium acetate (pH 4.0), 500 μl phenol, and 100 μl chloroform-isoamyl alcohol (49:1) were added to the homogenate with thorough mixing after each addi-

tion. The final suspension was mixed vigorously for 10 seconds and placed on ice for 15 min. Following centrifugation at 10,000×g for 2 min, the aqueous phase was transferred to a fresh tube and one volume of isopropanol was added to precipitate RNA and stored at −20℃ for overnight. The precipitated total RNA was recovered by centrifugation (10,000× g for 20 min at 4℃). The RNA pellet was dissolved in 150 μl of the homogenizing solution and precipitated with one volume of isopropanol at −20℃ for 3hr. The RNA was again recovered by centrifugation, washed twice with 75% ethanol, dried and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). The quantity of the extracted total hypothalamic RNA was determined by spectrophotometry (OD 260) and the quality of RNA was routinely checked by 1% agarose gel electrophoresis. The total hypothalamic RNA from one rat was 50~100 μg.

2. Determination of AVP and OT mRNA

Rat cDNA specific for the AVP and OT gene precursor was used for preparation of both antisense and sense AVP and OT RNA. Hypothalamic AVP and OT mRNA was determined by a modified method[5,6] of solution hybridization[9]. Briefly, hybridization was carried out in a reaction mixture containing 600 mM NaCl, 4 mM EDTA, 10 mM Tris, pH 7.5, 46% formamide, ^{35}S-asRNA (antisense strand RNA) as a probe (3000 cpm/tube), and 1 μg sample (total extracted RNA) in a final volume of 30 μl in an Eppendorf tube. The reaction solution was covered with 100 μl paraffin oil to prevent evaporation and incubated at 68℃ for 16 hr. Each hybridization included a standard curve consisting of 1, 2, 4, 8, 16, 32, and 64 pg AVP sRNA (AVP-sense strand RNA) or 3.9, 7.8, 15.6, 31.3, 62.5, 125, and 250 pg OT sRNA (OT-sense strand RNA). Each standard or sample was hybridized in triplicate. After hybridization, 300 μl of ribonuclease buffer (150 mM NaCl, 30 mM Tris-HCl, pH 8.0, 2 mM EDTA, 25 μl/ml RNase A, and 7 μl/ml RNase T1) was added to remove any unhybridized probe. Then the reaction mixture was incubated at 37℃ for 1 hr and terminated by addition of 300 μl of ice-cold 10% trichloroacetic acid (TCA). After vortexing, the precipitated samples were separated by filtration using 2.4 cm Whatman GF/C filters. The filters were counted in 5 ml scintillation fluid. Both ^{35}S-labeled asRNA (probe) and AVP sRNA (standard) were produced by in vitro transcription using rat AVP cDNA and Riboprobe Transcription System. AVP cDNA has 230 base pairs and contains the 3' end of the AVP cDNA. This cDNA is specific for the AVP precursor and shows no cross hybridization with the oxytocin precursor[10,11]. The in vitro transcribed cRNA probe corresponds to the sequence complementary to a Pst I-Dra I fragment of rat AVP cDNA and corresponds to the AVP specific part of the AVP mRNA. In the standard curve, hybridization was linear up to 64 pg of sRNA (Fig. 1). Plasma AVP levels were measured by radioimmunoassay as described earlier[12]. Plasma sodium, plasma osmolality, creatinine and blood urea nitrogen were determined by standard laboratory methods.

The data were analyzed by Student's "t" test.

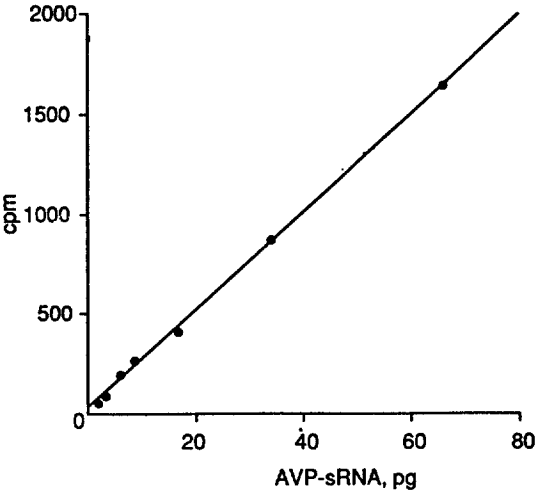

Fig. 1. Standard curve of AVP mRNA measurement by solution hybridization.

RESULTS AND DISCUSSION

Plasma osmolality of pregnant rats was 294.91±1.08 mOsm/kg which was 13.8 mOsm/kg lower than the age-matched virgin rats (308.70±1.16 mOsm/kg, $p<0.0001$, n=10, Fig. 2). But there was no significant difference in plasma AVP concentrations (Control (C): 2.62±0.46 pg/ml vs Pregnancy (P): 1.91±0.31 pg/ml, *NS*, n=10, Fig. 2). Hypothalamic AVP mRNA levels determined by solution hybridization method showed no significant difference either between the groups (C: 877.28±122.71 vs P: 1148.32±101.68 pg/hypothalamus, *NS*, n=10, Fig. 2). On the other hand, hypothalamic OT mRNA was significantly higher in the pregnant rats than the control rats (C: 3287.9±759.3 vs P: 7651.5±652.2 pg/hypothalamus, $p<0.0004$, n=10, Fig. 3).

The above findings suggest continued hypothalamic AVP biosynthesis in spite of lowered plasma osmolality. Hypoosmolality in rats induces downregulation of AVP biosynthesis and the synthesis is completely turned off by 7 day[13]. Plasma osmolality measured on pregnancy day 14 was already significantly lower than the age-matched controls (C: 315.67±1.90 vs P: 308.09±2.16 mOsm/kg, $p<0.019$, n=9). Hence, for the sustained hypothalamic AVP biosynthesis, there must be some nonosmotic stimulus. Since extracellular fluid volume is already expanded by more than 30~50%[14], the only possible explanation of the above findings is by the increase in the holding capacity of the arterial tree, namely primary arterial vasodilation.

Van Tol, et al[15] and Zingg, et al[16] reported parallel increase in AVP and OT mRNA levels from hypothalamus of 21-day pregnant rats by northern and dot blot analysis. Because they used synthetic oligonucleotide probes of 27 oligomers, it may be difficult to exclude the possiblity of cross reactivity.

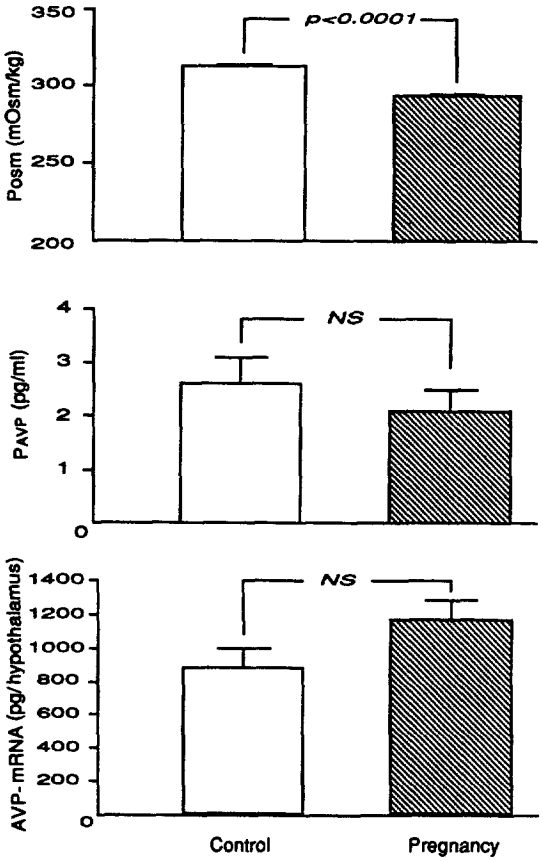

Fig. 2. Oxytocin mRNA in hypothalamus from control and pregnant (day 20) rats. Each bar represents mean±S.E.M., n=10.

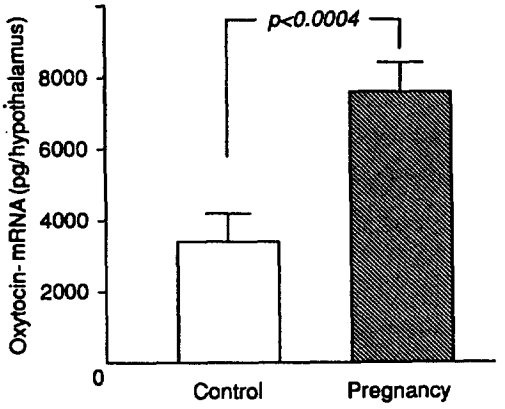

Fig. 3. Plasma osmolality (Posm), plasma AVP (P_{AVP}) and AVP mRNA of control and pregnant (day 20) rats. Each bar represents mean±S.E.M., n=10.

As mentioned above, the cDNA probe used in the present study is specific for the AVP precursor gene and shows no cross hybridization with the oxytocin precursor gene. Furthermore, since they sacrificed the rats on the day of labor, the close proximity of time of sacrifice and the labor could have affected the results. Anyway, with this highly specific and sensitive method. we could not reproduce the parallel increase in AVP and OT mRNA levels in pregnant rats. But OT mRNA increased more than 2 fold in 20-day pregnant rats.

From the above results, it is concluded that hypothalamic AVP biosynthesis continues despite hypoosmolality and primary arterial vasodilation may be responsible for the sustained biosynthesis and release of AVP.

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