Effects of Environmental Temperature on Atrial Natriuretic Peptide (ANP)–granules of Auricular Cardiocytes and Plasma ANP Level in Pregnant Rats

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The atrial natriuretic peptide (ANP) levels of auricular cardiocytes and plasma were examined by immunohistochemistry, ultrastructural morphometry, and radioimmunoassay (RIA) in pregnant rats (4th, 12th, 20th day of gestation) under 22°C and 33°C environmental conditions. Immunohistochemically, ANP immunoreactivity was stronger on the 20th day of gestation in the 22°C environmental groups, but weaker on the 12th and 20th days of gestation in the 33°C environmental groups. According to the results of ultrastructural morphometry, the number of ANP-granules had increased significantly by the 20th day of gestation in the 22°C environmental groups, but was decreased on the 12th and 20th days of gestation in the 33°C environmental groups. RIA demonstrated that the maternal plasma ANP concentration decreased gradually during pregnancy in the 22°C environment. The plasma ANP concentration in the 33°C environmental groups tended to be lower than that at the same stage of pregnancy in the 22°C environmental groups. — KEY WORDS: atrial natriuretic peptide, cardiocyte, environmental temperature, pregnant rat

It is well known that atrial cardiocytes in mammals are endocrine cells which secrete atrial natriuretic peptide (ANP) [1-3] which has diuretic, natriuretic and vasodilatory properties and exerts an inhibitory action on aldosterone, cortisol, arginine vasopressin and renin release. Also, ANP is a peptide hormone believed to be involved in blood pressure and volume homeostasis [6, 7].

On the other hand, Yamauchi et al. [18], and Fujita and Yamauchi [4] reported that approximately 50% of pregnant rats died on days 18–20 of gestation under hot environmental conditions with indications of hypertension. They suggested that the death of pregnant rats in a hot environment may be caused by hypertension, while the mechanism by which hypertension caused death in these pregnant rats remains obscure. In the present study, therefore, changes in ANP circulation were examined during pregnancy under different environmental conditions to obtain fundamental data providing explanations as to the etiology of hypertension in pregnant rats in hot environments.

Hearts were obtained from 5 pregnant rats of the Wistar strain (Jcl: Wistar, CLEA Japan, Inc., Osaka, Japan) in each group on the 4th, 12th or 20th day of gestation under different environmental conditions. The day of
insemination was defined as the 0-day of gestation, and control groups were kept in a normal environment room (temperature: 22±1°C; humidity: 55±5%; automatic lighting: 6:00 to 20:00). Other groups were transferred to the hot environment room (temperature: 33±1°C; humidity: 55±5%; automatic lighting: 6:00 to 20:00) on the day of insemination. All animals were kept in polycarbonate cages with autoclaved wood shavings, and fed a pellet diet CE-2 (CLEA Japan, Inc., Osaka, Japan) and water ad libitum. Auricular tissues (distal part of the atrium) were removed from these animals under Nembutal anesthesia. For immunohistochemical examinations, right auricular tissue blocks were fixed with Zamboni’s solution for 24 hr at room temperature. Immunohistochemical staining was performed according to the modified avidin–biotin–peroxidase complex (ABC) technique described in our previous report [12]. The antibody was raised in rabbits against synthesized human atrial natriuretic peptide/cardiodilatin (hANP/hCDD, 99-126) [14,15], and diluted 1:1,000 for use. For ultrastructural morphometry, tissue blocks of the right auricle were fixed in 2% paraformaldehyde-2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 and post-fixed in 1% osmium tetroxide in the same buffer. Thin sections were double-stained with uranyl acetate and lead citrate and examined with a JEM-2000 EX electron microscope. The number of ANP-granules in right auricular cardiocytes was measured according to our previous methods [9,10]. In each gestation group, the means (±SD) of counts were calculated in 50 photographs from 5 pregnant rats and statistically analyzed by Student’s t test. The plasma ANP concentration in each gestation group was measured by radioimmunoassay (RIA), as described in our previous report [11].

Table 1. Comparisons of the numbers of ANP-granules in right auricular cardiocytes of pregnant rats under different environmental temperatures (22°C, 33°C) (Mean±SD)

<table>
<thead>
<tr>
<th>Day of gestation</th>
<th>Environment</th>
<th>22°C</th>
<th>33°C</th>
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<tbody>
<tr>
<td>4th</td>
<td>122.3±12.1</td>
<td>115.6±11.3</td>
<td></td>
</tr>
<tr>
<td>12th</td>
<td>118.4±10.6</td>
<td>68.4±8.7**</td>
<td></td>
</tr>
<tr>
<td>20th</td>
<td>175.5±15.6*</td>
<td>61.5±9.2**</td>
<td></td>
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</tbody>
</table>

* significantly different from the 4th or 12th day of gestation in the 22°C environment, and all pregnant groups in the 33°C environment (P<0.01)
** significantly different from 4th day of gestation in the 33°C environment and all pregnant groups in the 22°C environment (P<0.01)

of gestation (Figs. 1a, b). On the 4th day of gestation, the ANP immunoreactivity did not differ between the 22°C and the 33°C environmental groups, but was weaker on the 12th and 20th days of gestation than on the 4th day of gestation in the 33°C environmental groups (Figs 1c, d). There was no difference in the reaction between the 12th and 20th days of gestation in 33°C environmental groups.

Ultrastructural morphometry was used to analyze the number of ANP-granules in right auricular cardiocytes, as shown in Table 1. In the 22°C environmental groups, the number of ANP-granules was significantly greater on the 20th day of gestation than on the 4th and 12th days of gestation (P<0.01). In the 33°C environmental groups, the number of ANP-granules was significantly less on the 12th and 20th days of gestation than on the 4th day of gestation (P<0.01). There was no significant difference in the number among the 4th and 12th days of gestation under the 22°C environmental conditions and the 4th day of gestation in the 33°C environment.

RIA results for maternal plasma ANP levels (mean±SE) in 22°C environmental groups were as follows: the 4th day of gestation, 102.6±14.3 fmol/ℓ (n=4), the 12th day of gestation, 55.5±14.5 fmol/ℓ (n=5), the 20th day of gestation, 69.7±8.3 fmol/ℓ (n=9). The maternal plasma ANP concentration gradually decreased during pregnancy in the 22°C environment. In the 33°C environmental groups, the plasma ANP levels were as follows: the 4th day of gestation, 63.2±8.1 fmol/ℓ (n=5), the 12th day of gestation, 38.5±21.8 fmol/ℓ (n=5).
4), the 20th day of gestation, 39.2±13.5 fmol/l (n=7). The plasma ANP concentration in the 33°C environmental groups tended to be lower than that at the same stage of pregnancy in the 22°C environmental groups.

Circulating ANP levels during normal pregnancy in the rat [8,13] goat [16], and human [17] have been reported by several authors. During normal pregnancy, the plasma ANP level was suggested to be undergoing a gradual increase due to increased blood volume and cardiac output [17]. Another report demonstrated that circulating ANP levels tended to decline during pregnancy, but were not significantly different from those observed in the nonpregnant state [13]. In this study, plasma ANP levels tended to decline gradually during pregnancy under 22°C environmental conditions. Morphologically, the number of ANP-granules and immunoreactivity for ANP in cardiocytes were significantly increased in the final period of pregnancy (20th day of gestation) in the 22°C environment. These findings may represent an increase in the stores of propeptides or of precursor forms of ANP, in cardiocytes, as a result of reduced ANP secretion during pregnancy. On the first postpartum day, the plasma ANP level is increased due to a marked ANP release at delivery [13]. Thus, the increase in ANP-granules in cardiocytes at the end of pregnancy may be associated with physiological control aimed at releasing a large amount of ANP up on delivery.

In this study, the morphological findings under 33°C environmental conditions presented a striking contrast to those in the 22°C environment. Furthermore, plasma ANP levels in the 33°C environment tended to be lower than those at the same stage of pregnancy under 22°C environmental conditions. The death of pregnant rats under 33°C environmental conditions may be related to a decrease in ANP release upon delivery due to a reduction in levels of circulating ANP during the final part of the pregnant period, as compared to that during a similar period in the 22°C environment. The appearance of hypertension in pregnant rats in the 33°C environment reported by Fujita and Yamauchi [4,5] may be related to reduced levels of circulating ANP during pregnancy.

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References

妊娠ラットにおける心房性ナトリウム利尿ペプチド(ANP)顆粒ならびに血漿中ANP濃度に及ぼす環境温度の影響

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22℃および33℃飼育下における妊娠4, 12, 20日目のラットの心房性ナトリウム利尿ペプチド(ANP)顆粒を免疫組織化学的ならびに形態計測的に、血漿ANP濃度をラジオイムノアッセイにより解析した。免疫組織化学的に、22℃飼育群のANP免疫反応は妊娠20日目で強くなったが、33℃飼育群においては、妊娠12, 20日目で弱くなった。形態計測的に、22℃飼育群ではANP顆粒の数は、妊娠20日目に有意に増加していたが、33℃飼育群では妊娠12, 20日目に有意に減少していた。血漿中のANP濃度は、22℃飼育群において妊娠日齢と共に減少し、33℃飼育群では22℃飼育群の同時期に比べ低い傾向にあった。

Explanation of Figure

Fig. 1. Immunohistochemical staining of ANP in right auricular cardiocytes. All magnifications are × 314. ANP immunoreaction is localized primarily in the perinuclear region (arrow heads). The reaction is stronger on the 20 th day of gestation (a) than on the 4 th day of gestation (b) in the 22°C enviromental groups. The ANP immunoreactivity is weaker on the 20 th day of gestation (c) than on the 4 th day of gestation (d) in the 33°C environmental groups. On the 4 th day of gestation (b, d), there is no difference in reactivity between the 22°C and the 33°C environmental groups.
Fig. 1.