Increased amniotic vasopressin levels in experimentally growth-retarded rat fetuses

H. P. Oosterbaan* D. F. Swaab & G. J. Boer**

*Groot Ziekenhuis, Dept. of Obstetrics and Gynaecology, Nieuwstraat 34, 5211 NL's Hertogenbosch, The Netherlands and **Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam ZO, The Netherlands

Received 20th June, 1984 and in final form 11th October, 1984

Abstract

Arginine-vasopressin (AVP) and oxytocin are neuropeptides that are not only released as hormones into the peripheral circulation, but are also involved in central processes, e.g., in brain development. Earlier experiments suggested an inverse relationship between amniotic AVP and fetal growth. To see whether increased peptide levels reflect fetal growth retardation, and to determine cause and effect of this relationship, AVP and oxytocin content were determined in amniotic fluid of growth-retarded fetuses by radioimmunoassay. Growth retardation was established either by intraperitoneal administration of methylazoxymethanol to the mother, or by undernourishment of the mother. Elevated amniotic AVP levels were found in the methylazoxymethanol-treated and undernourished rats, partly concomitant with smaller amount of amniotic fluid. Amniotic AVP levels were inversely related to fetal body weight, while a similar trend was found for fetal brain weight. In addition, a positive correlation was found between fetal body weight and amniotic oxytocin in control rats.

Introduction

Arginine-vasopressin (AVP) and oxytocin are peptides produced by neurons in the hypothalamus and transported to the neurohypophysis, from which they are released into the peripheral circulation. In addition to this classical neurosecretory pathway, exohypothalamic fibres that are in synaptic contact with other neurons in different brain areas (Buijs & Swaab, 1979) have been demonstrated immunocytochemically. Apart from their well-known functions in body water regulation, lactation and labour, these peptides seem to be involved in a number of central processes (Swaab, 1982). Since smaller brains are found in the vasopressin-deficient Brattleboro rat, brain development might be one of those processes (Boer, Van Rheenen-Verberg & Uylings, 1982). The combination of properties of the neuropeptides AVP and oxytocin (1) both as hormones and as centrally active compounds, (2) their early presence in development (Swaab & Ter Borg, 1981), (3) and their possible effects during brain development, led us to investigate the question whether they might be used to monitor disturbances in fetal development. If changes in amniotic fluid would be related to changes in fetal development, such neuropeptides might become suitable as functional parameters for indicating a retardation in fetal brain development in clinics as well.

*Correspondence to H. P. Oosterbaan
In a previous study, in which we investigated the passage of AVP and oxytocin from the mother to the amniotic fluid in the rat, we observed a highly significant inverse relationship between fetal body and brain weight with amniotic AVP content in AVP-loaded mothers (Oosterbaan, Swaab & Boer, 1985). It was suggested from that study that the increased AVP levels observed in amniotic fluid after loading the mothers with a high dosage of AVP, was not the result of a passage from the mother to the amniotic compartment, but rather a consequence of an increased secretion of AVP by the growth-retarded fetuses. The present study was, therefore, designed to see whether AVP and oxytocin levels in amniotic fluid and maternal blood were influenced by growth retardation in rat fetuses. Since each method of inducing fetal growth retardation has its own advantages and disadvantages, this condition was brought about by two different methods, including repeatedly administered intraperitoneal injections of methylazoxymethanol to the mother (Haddad, Rabe, Lacqueur, Spatz & Valsamis, 1969) and undernourishment of the mother (Patel, Balazs & Johnson, 1973).

**Materials and Methods**

**General procedures**

Virgin female Wistar rats (n = 35) were obtained from CPB-TNO (Zeist, The Netherlands), weighing approximately 180 g. The rats were housed in groups at 25°C and exposed to 12 h light daily from 7 am to 7 pm. They had free access to food and tap water. Mating with corresponding males produced a total of 390 live fetuses. The day the vaginal smear was positive for spermatozoa was taken as day zero of pregnancy. (Under our laboratory conditions, delivery usually takes place late on day 21; Boer, Lincoln & Swaab, 1975.) Following the experimental procedures (see below), the pregnant rats were sacrificed by decapitation on day 20. Maternal trunk blood was collected in pre-chilled tubes containing 100 units of Na-heparin (Sigma) and plasma was separated after centrifugation at 1,500 g for 20 min at 20°C. A Caesarian section was performed and amniotic fluid was pooled in pre-chilled polyethylene tubes (Luckham), each containing the fluid of approximately 6 fetuses. The total volume of collected amniotic fluid was established by weighing the tubes, with a sensitivity of 0.01 mg. Living fetuses as well as placentas, from which the membranes and umbilical cords had been removed, were weighed with a sensitivity of 0.01 g after gentle blotting. The fetal brains were subsequently removed from the skulls and immediately weighed (sensitivity 0.01 mg), thus avoiding desiccation. Litters from which more than two fetuses had been found dead were excluded from the study.

**Methylazoxymethanol procedure**

The rats were divided randomly into two groups. On days 14, 15 and 16 of pregnancy the animals (n = 21) were injected intraperitoneally between 10 am and 1 pm, either with 20 mg/kg of methylazoxymethanol (Sigma lot no. M1009), diluted to a concentration of 10 mg/ml in 0.9% NaCl (Haddad et al., 1969) or with physiological saline only as control (n = 11).

**Undernutrition procedure**

From day 6 of pregnancy onwards females were housed individually and divided at random into two groups. The first group (n = 10) received a restricted food intake of approximately 50% (6.75 g/day) of the daily dietary needs (Am. II, Hope Farms), while the control group (n = 8) had free access to food. Both groups received water ad lib.

**Radioimmunoassay**

Both AVP and oxytocin were measured after Vycor extraction of the serum samples by highly specific radioimmunoassay as described earlier (Dogterom, Snijdwint, Pevet & Swaab, 1980). All assays were set up in duplicate. The limit of detection was 1 pg for oxytocin and 0.5 pg for AVP. The interassay coefficient of variation for AVP and oxytocin (after extraction of the
samples) was 13.9% and 8.6%, respectively, while the intra-assay coefficient of variation (for unextracted samples) was 15.7% and 10.2%, respectively. These data were all obtained with samples containing 16 or 32 pg.

**Statistics**
For calculation of differences for the various weights and volumes, Student's t-tests (two tailed) were used. The non-parametric Mann-Whitney-U test (two-tailed; corrected for ties) was used for the neuropeptide levels. The Spearman rank test was used for correlations. All calculations were performed by means of the Statistical Package of the Social Sciences (SPSS) and $P<0.05$ (two-tailed) was considered to be significant.

**Results**

**Litter sizes and survival rates**
Of the methylazoxymethanol-treated dams 62% died before day 20, whereas all undernourished and control females survived. Only one control mother of the undernutrition group had more than two dead fetuses and was therefore excluded from the study. The survival rates of all the fetuses used in the present study were high: 97% for both control and methylazoxymethanol-treated mothers and 98% for both control and undernourished mothers.

**Fetal weights and amniotic fluid volume**
In both experimental groups a significant decrease in fetal body, brain and placental weight was revealed as compared with control groups. A significant drop in amniotic fluid volume per fetus was found in both groups (Table 1).

**Maternal plasma and amniotic fluid AVP and oxytocin levels**
In both methylazoxymethanol-treated and undernourished animals maternal plasma AVP and oxytocin levels were not elevated as compared with control levels (Table 2). Amniotic AVP levels (pg/ml) were significantly higher than control levels in the methylazoxymethanol-treated and undernourished groups, while no change in amniotic oxytocin was found. To allow for differences in amount of amniotic fluid we also calculated the total amount of AVP or oxytocin present in the amniotic fluid per fetus (pg/fetus). Although in both groups the AVP levels per fetus were about twice as high in the experimental groups, only in the undernourished rats was the amount of AVP per fetus significantly higher (Table 2).

**Relationship between fetal weight and amniotic peptide levels**
No significant correlations were found in the control groups between amniotic AVP (average/fetus) and average fetal body weight, but in the group of undernutrition controls a significant inverse relationship between fetal brain weight and amniotic AVP was found ($r = 0.43; p = 0.046$).

Oxytocin content was positively correlated with fetal body weight in both control (methylazoxymethanol controls: $r = 0.41; P = 0.031$; undernutrition controls: $r = 0.54; P = 0.015$), while for the undernutrition controls, in addition, a significant positive correlation existed with fetal brain weight ($r = 0.50; P = 0.023$). No such correlations were seen in the experimental groups. In both experimental groups a significant negative correlation was found between amniotic AVP and fetal body weight (methylazoxymethanol: $r = -0.50; P = 0.048$; undernutrition: $r = -0.38; P = 0.048$), while a similar trend was seen for fetal brain weight.
Table 1  Effects of fetal body, brain and placental weight and mean amniotic fluid volume of maternal undernutrition of methylazoxymethanol treatment.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Experimental</th>
<th>% Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>Methylazoxymethanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>undernutrition</td>
<td>3.38 ± 0.03(127)</td>
<td>1.85 ± 0.07(63)**</td>
<td>45</td>
</tr>
<tr>
<td>undernutrition</td>
<td>3.34 ± 0.03(84)</td>
<td>2.17 ± 0.03(117)**</td>
<td>35</td>
</tr>
<tr>
<td><strong>Brain weight (g)</strong></td>
<td>Methylazoxymethanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>undernutrition</td>
<td>0.166 ± 0.001(127)</td>
<td>0.105 ± 0.002(63)**</td>
<td>37</td>
</tr>
<tr>
<td>undernutrition</td>
<td>0.162 ± 0.001(84)</td>
<td>0.132 ± 0.001(117)**</td>
<td>19</td>
</tr>
<tr>
<td><strong>Placental weight (g)</strong></td>
<td>Methylazoxymethanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>undernutrition</td>
<td>0.49 ± 0.01(127)</td>
<td>0.38 ± 0.01(63)**</td>
<td>23</td>
</tr>
<tr>
<td>undernutrition</td>
<td>0.51 ± 0.01(84)</td>
<td>0.36 ± 0.01(117)**</td>
<td>41</td>
</tr>
<tr>
<td><strong>Amniotic fluid volume (μl)</strong></td>
<td>Methylazoxymethanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>undernutrition</td>
<td>320 ± 10(22)</td>
<td>215 ± 15(12)**</td>
<td>33</td>
</tr>
<tr>
<td>undernutrition</td>
<td>330 ± 10(20)</td>
<td>295 ± 10(16)*</td>
<td>10</td>
</tr>
</tbody>
</table>

Results are means ± SEM with number of measured values in parentheses. P values for comparison of experimental and control groups are: * <0.025; ** <0.001
Table 2  Maternal and amniotic AVP and oxytocin levels after maternal undernutrition of methylazoxymethanol treatment

<table>
<thead>
<tr>
<th></th>
<th>AVP</th>
<th>Oxytocin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Experimental</td>
</tr>
<tr>
<td>Maternal plasma (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylazoxymethanol</td>
<td>3 ± 1.8(11)</td>
<td>12 ± 5.0(6)</td>
</tr>
<tr>
<td>Undernutrition</td>
<td>22 ± 28.7(7)</td>
<td>13 ± 26.1(9)</td>
</tr>
<tr>
<td>Amniotic fluid (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylazoxymethanol</td>
<td>12 ± 10.8(22)</td>
<td>40 ± 26.9(12)*</td>
</tr>
<tr>
<td>Undernutrition</td>
<td>8 ± 11.1(16)</td>
<td>24 ± 6.4(20)**</td>
</tr>
<tr>
<td>Total amniotic (pg/fetus)‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylazoxymethanol</td>
<td>3.6 ± 3.7(21)</td>
<td>8.4 ± 5.4(12)</td>
</tr>
<tr>
<td>Undernutrition</td>
<td>2.8 ± 3.9(14)</td>
<td>7.6 ± 2.6(19)*</td>
</tr>
</tbody>
</table>

Results are means ± SEM with number of values in parentheses. P values are: * < 0.01; ** <0.001, all others >0.05. ‡ = median values are calculated from concentration data (pg/ml) by multiplying with the ratio total amniotic volume: number of pooled pups from which this volume is derived.
Discussion

Methylazoxymethanol treatment proved to be highly toxic for the dams. However, if they survived, no excess fetal loss was found in the litters. Therefore, this model was still useful. Initially fetal growth retardation by unilateral ligation of the uterine vessels on day 16 of pregnancy (cf. Wigglesworth, 1964) was tried. In spite of the fact that this is the most common method obtaining intrauterine growth retardation (Evans, Mukkerjee & Schulman, 1983), it was abandoned because of high fetal loss (64%).

Following both methylazoxymethanol treatment and undernutrition a significant growth retardation was achieved. Methylazoxymethanol is a potent antimitotic agent that acts on dividing cells, presumably by methylating nucleic acids (Nagata & Mashumoto, 1969). The retardation of brain growth following methylazoxymethanol is considered to be mainly the result of a reduction of the number of neurons (Haddad et al., 1969; Johnston, Carman & Coyle, 1981). Undernutrition is supposed to give a more generalized pattern of growth failure, which, at least in part, is the result of a reduced placental transfer of nutrients (Rosso, 1977a,b). Maternal food deprivation leads to a reduced protein and DNA content in the fetal brain (Zamenhof, Marthens & Margolis, 1968).

Haddad et al. (1969) observed an overall decrease in brain weight of approximately 30%, after i.p. injection of methylazoxymethanol, while in our group the decrease was 37%. In the undernutrition group on day 20 we noticed a decrease of some 35% in body weight, which is identical to the findings of Patel et al. (1973), while fetal brain weights were less severely affected. In conclusion, the methylazoxymethanol experiment leads to a type of stunted growth in which body and brain growth are symmetrically decreased, while undernutrition treatment gives rise to a more asymmetrical growth retardation in which brain sparing can be observed (Evans et al., 1983).

In the growth-retarded groups a decrease in amniotic fluid volume was observed following growth retardation, a phenomenon that is often concomitant with human pregnancies complicated by dysmature intrauterine growth (Manning, Hill & Platt, 1981). Although smaller fetuses may produce a smaller amount of amniotic fluid, a reduced body size alone is not a sufficient explanation for a reduction in the amniotic fluid volume. For instance, at term the amount of amniotic fluid in the rat falls, while the fetal body weight rises considerably. Hypophysial hormones like AVP and prolactin are thought to play a role in the regulation of the amniotic fluid balance (Perks & Vizsolyi, 1973; Perks, Vizsolyi, Holt & Cassin, 1978). However, our present and previous data do not reveal an unequivocal relationship between the amniotic neurohypophysial hormone level and the changes in the amount of amniotic fluid. The present data reveal that higher amniotic AVP levels are present in growth-retarded fetuses in combination with lower amounts of amniotic fluid. This is at variance with Perks’ work, which suggests that elevated amniotic AVP levels would result in an increased amount of amniotic fluid. In addition, in human and rat anencephalics, which are often associated with hydramnios, no AVP can be demonstrated in the amniotic fluid (Honnebier & Swaab, 1973; Swaab & Oosterbaan, 1983). Finally, a normal amount of amniotic fluid was present in homozygous, i.e., vasopressin-deficient Brattleboro rat fetuses (Oosterbaan et al., 1985). During stunted fetal growth AVP might reduce amniotic fluid production indirectly by redistributing
the fetal circulation under conditions of chronic stress (see also below). In hypoxia and growth retardation in fetal sheep the renal blood flow falls dramatically while pulmonary blood flow almost comes to a standstill (Cohn, Sacks, Heyman & Rudolph, 1974). Fetal urine and lung fluid are considered to be the main sources for amniotic fluid in late pregnancy (Abramovitch, 1978). In addition, AVP was demonstrated to cause a passage of water across the fetal skin of guinea pig in the direction of the fetus (Perks, 1977), thus possibly also reducing the amount of amniotic fluid.

The observed rise in amniotic fluid AVP following growth retardation is smaller when expressed per total amount present in the amniotic fluid per fetus (Table 2). Yet, the increased AVP levels cannot simply be explained by the reduced amounts of amniotic fluid in growth retardation. The oxytocin levels remain unchanged, and the ratio of AVP/oxytocin in amniotic fluid rises from 0.33 and 0.32 in controls to 1.04 and 0.8 in the growth-retarded groups (undernutrition and methylazoxymethanol, respectively).

One might expect a decrease in amniotic neurohypophyseal hormone levels in growth retardation. A stunted brain development might be accompanied by a stunted development of the hypothalamo-neurohypophysial system. Thymidine labelling studies demonstrate that the formation of the cells of the paraventricular nuclei and supraoptic nuclei in the rat takes place between days 12–13 and 15–16 of gestation (Altman & Bayer, 1978), and methylazoxymethanol treatment results in a reduction in the total number of neurons (Johnston et al., 1981).

Under circumstances of stress AVP is thought to promote adaptation by redistribution of the blood flow in favour of the vital organs such as the brain, adrenals, heart and uteroplacental circulation (Iwamoto, Rudolph, Keil & Heymann, 1979; Pohjavuori & Fyhquist, 1980). AVP might also be released as a corticotropic hormone (Gillies, Linton & Larry, 1982), thus stimulating the fetal adrenal to mature faster (Becker & Becker, 1976), and therefore be instrumental in e.g., the stimulation of fetal lung maturation. The hypothesis that fetal growth retardation acts as a stress that increases secretion of AVP by the fetus, is supported by the observed significant inverse correlations between fetal body weight and amniotic AVP. This observation is in line with results from our earlier experiment, in which mothers were given a high dosage of AVP resulting in stunted fetal growth (Oosterbaan et al., 1985). At present it is not known whether the inverse relationship between amniotic AVP and fetal brain weight, as found in the controls of the undernourished group, is also due to some control fetuses being in an unfavourable condition.

In contrast to the inverse relationship between amniotic AVP and fetal growth in methylazoxymethanol-treated and undernourished rats, both control groups showed a positive correlation between fetal body weight and amniotic oxytocin. In addition, in the control rats of the undernutrition group, amniotic oxytocin was positively related to fetal brain weight. In conclusion, it therefore seems that an elevation of AVP in amniotic fluid might reflect an unfavourable condition of the fetus and indicate a disturbed fetal brain growth, while a rise in amniotic oxytocin might reflect normal fetal brain development.

Acknowledgments

The authors wish to thank Tjitske van der Woude and Bart Fisser for their assistance in the operation procedures, Joop van Heerikhuize for doing the assays and Peter van Nieuwkoop for his secretarial help. This study was made possible by grants from the Van den Houten Fund and the Committee for the Furtherance of Veterinary and Comparative Pathological Research.
References


