Developmental Changes in the Umbilical Arteries with Observation of the Effects of Indomethacin in Fetal Rats

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ABSTRACT. The caliber of the umbilical arteries was measured in fetal rats on day 14 to 21 of gestation. The fetuses were rapidly frozen in an acetone-dry ice mixture. The caudal half of each whole-body frozen fetus was gradually shaved with a knife from the ventral side to expose the umbilical arteries for measurement of their calibers. Observation reveals that, on day 15 of gestation, the umbilical arteries are asymmetrical in that the right artery is more dilated than the left. It is further seen that the left umbilical artery is closed on day 17 in contrast to the right which is increasingly more dilated toward term. When indomethacin, a synthetic compound having an action similar to that of glucocorticoids, was administered to pregnant rats 3 hr prior to sacrifice on day 21 of gestation, the right fetal umbilical artery was significantly constricted. The reason for this phenomenon is unknown, but is discussed in association with the manner of closure of the ductus arteriosus.—KEY WORDS: fetal rat, indomethacin, umbilical artery, whole-body freezing.

During the prenatal period, the umbilical arteries play an important role in conveying carbon dioxide and waste products from the fetus to the placenta. After birth, with the loss of placental circulation, the umbilical arteries close to remain as cord-like ligaments running along either side of the bladder and the intra-abdominal midventral line toward the umbilical part of the abdomen.

Regarding the developmental changes in the umbilical arteries, the rat has received only fragmentary study, but it has been observed that the left umbilical artery is already vestigial at the end of intra-uterine life [8].

In addition to the umbilical artery, the ductus arteriosus, which is dilated by prostaglandins, also plays an important role in prenatal life, shunting most of the blood from the pulmonary artery to the aorta [1, 2, 5]. Both in the human and in the rat, it has been shown that the ductus arteriosus is constricted a few hours after maternal administration of glucocorticoid hormones in the preterm infant [6, 7]. This phenomenon may be due to the inhibitory action of glucocorticoid hormones upon the biosynthesis of prostaglandins [3, 4].

The present work was designed to observe the chronological process of closure of the umbilical artery in more detail in fetal rats, with examination of the effects induced upon the arterial closure by maternal administration of indomethacin, a synthetic compound having an action similar to that of glucocorticoid hormones, and which is effective at a small dosage. The method used here for exposure of the fetal umbilical arteries is unique in its way of shoving and examining whole-body frozen fetuses directly by the use of a surgical knife under a dissecting microscope.
MATERIALS AND METHODS

Female Wistar rats, 12–15 weeks old at the time of mating, were used in this work. They were maintained on a 12 hr light, 12 hr dark cycle, with food (Labo-MR-Breeder) and water *ad libitum*, and kept at a room temperature of 22±3°C and humidity of 55±10%. The females were placed with males overnight and examined the next morning for the presence of sperm in the vaginal smear. The day on which sperm was found was designated as day 0 of gestation, and the females were caged individually at this time.

Observation and calibration of the fetal umbilical arteries were performed on days 14 through 21 of gestation. After caesarian section, each fetus was rapidly immersed in an acetone-dry ice mixture. In order to minimize errors due to differences in fetal body size, frozen fetuses were individually weighed, and then 4 fetuses of similar weight were selected from each litter and stored for several days at −20°C prior to observation. At the time of observation, the upper half of each fetal body cranial to the umbilical area was removed, and the remaining half of the body was placed on the freezing plate of a thermo-electric freezing unit (Komatsu Electric Inc., Tokyo), oriented so that the dorsal side was down and the cranial end was slightly elevated, the orientation being adjusted by addition of water drops beneath the fetus (Fig. 1). With a surgical knife, under a dissecting microscope, the body was carefully shaved.

Fig. 1. Diagrammatic representation of a fetal body placed on the freezing plate, showing the levels at which the umbilical arteries were observed. A: the level at which the section of the bladder is the largest, B: the level at which the umbilical artery bends towards the bladder, BL: bladder, UA: umbilical artery, UV: umbilical vein, W: frozen water drop, *: pubic symphysis.

Fig. 2. Section exposed by shaving down from the umbilical ring (day 18). The figure is oriented with the cranial side uppermost. UA: umbilical artery, UV: umbilical vein. ×11.
Fig. 3. Section exposed by further shaving, showing the bladder (BL) in the center. ×11.
Fig. 4. Section exposed by further shaving, showing the testis (T) and the pubic symphysis (PS). ×11.
from the ventral side toward the back. By shaving a little beyond the umbilical ring, sections of umbilical arteries and veins together with two vessels running toward the mesentery appeared (Fig. 2). By further shaving with careful viewing of the umbilical arteries, there successively appeared sections of the intestines, the top of the bladder and the pubic symphysis (Fig. 3). At the point where the pubic symphysis was nearly shaved off and when the section of the bladder became the largest (Fig. 4), the shaving was stopped in order to measure the caliber of the left and right umbilical arteries (level A in Fig. 1).

In order to examine the effects of indomethacin on the umbilical arteries, rats were used on day 21 of gestation. Maternal administration of indomethacin was carried out 3 hr or 9 hr prior to sacrifice. Indomethacin (Sigma Chemical Co.) was dissolved in saline, at a concentration of 1 mg/10 ml, and was given orally to each pregnant rat, by stomach tube, at a dose of 1 mg/kg body weight. As controls, other rats were given saline alone. In these experiments, calibration of the umbilical arteries was made at the two levels; one was the level where the arteries began to bend toward the bladder (B in Fig. 1) and the other was as described above (A in Fig. 1). These measurements were carried out to know whether there was any difference in constriction between these two levels, proximal (A) and distal (B). In the experiments with indomethacin, 3 to 6 fetuses per litter were used.

Statistical analyses of data were made with Student's t-test. A P value less than 0.05 was considered to be statistically significant.

RESULTS

Developmental changes in the umbilical arteries: The results of measurements in untreated rats are shown in Fig. 5. Since the caliber of the umbilical artery was not different between male and female, the data are summarized for both sexes. On day 14 of gestation, the right and left umbilical arteries (RUA and LUA) were almost the same in size, and were filled with blood (Fig. 6). On day 15, the RUA was slightly but significantly dilated as compared with the LUA which remained about the same as one day earlier. On day 16, the RUA was further dilated, while the LUA was extremely constricted, but still contained blood (Fig. 7). On day 17, in contrast to the enlarged RUA, the LUA was nearly closed with almost no blood inside and was apparently flattened from side to side (Fig. 8). Throughout the following days, the RUA became more and more dilated, while the LUA remained as a cord-like structure, becoming thinner day by day until day 21 when it became difficult to visualize the LUA under the dissecting microscope (Fig. 9).

![Fig. 5. Changes in the caliber of the fetal umbilical arteries, at level A, from day 14 to day 21 of gestation. Each value is the mean of 12 fetuses from 3 litters. Open circle: umbilical artery, closed circle: left umbilical artery, each vertical bar indicates standard error of the mean, *: significantly different from the value one day earlier (P<0.05), #: significantly different between right and left values on the same day (P<0.05).]
Fig. 6. Day 14. RUA: right umbilical artery, LUA: left umbilical artery. ×16.

Fig. 7. Day 16. The RUA is enlarged, while the LUA is constricted. ×16.

Fig. 8. Day 17. The LUA is flattened from side to side. ×16.

Fig. 9. Day 21. The LUA is not observable. ×16.

Fig. 10. Section at level B of a fetus in the indomethacin-treated group given indomethacin 3 hr prior to sacrifice on day 21 of gestation. The RUA is slightly constricted, as compared with that in a control fetus in Fig. 11. ×16.

Fig. 11. Section at level B of a control fetus given saline alone. ×16.

Table 1. Changes in the caliber of the umbilical artery following maternal treatment with indomethacin 3 or 9 hr prior to sacrifice on day 21 of gestation

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of fetuses</th>
<th>No. of litters</th>
<th>Caliber of umbil. art.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level A (µm)</td>
<td>Level B (µm)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>28</td>
<td>5</td>
<td>447±10</td>
</tr>
<tr>
<td>3 hr</td>
<td>18</td>
<td>5</td>
<td>417±15 ns</td>
</tr>
<tr>
<td>9 hr</td>
<td>30</td>
<td>5</td>
<td>431± 9 ns</td>
</tr>
</tbody>
</table>

Levels A,B: refer to the text.
*: significantly different from the control (p<0.01).
ns: not significant.
Effects of indomethacin: The results are presented in Table 1. In the group given indomethacin 3 hr prior to sacrifice, the RUA was significantly reduced in caliber at level B and its wall appeared whitish, as compared with the control (cf. Figs. 10, 11). At level A, there was no difference between the experimental group and the control group. In the group given indomethacin 9 hr prior to sacrifice, at neither level did the RUA differ from that in the control.

DISCUSSION

In the present work, the whole-body freezing method and the direct exposure of the umbilical arteries by shaving the body of the frozen fetus yielded good preparations for observation of developmental changes in the caliber of the arteries, with only a minimum of artifacts and a minimum of time lag in preserving the in vivo conditions.

The foregoing observation reveals that, on day 15 of gestation in fetal rats, the right and left umbilical arteries are asymmetrical such that the right is more dilated than the left. The observation further reveals that the left artery is closed on day 17 of gestation in contrast to the right which continues to enlarge throughout gestation. In the present study, only 9 of 830 specimens examined (1.1%) showed a reverse situation (the right closed and the left dilated). These were excluded from the present data. No cases showed both umbilical arteries patent. In any event, closure occurs on one side while dilation proceeds on the other side.

As described in the introduction, the ductus arteriosus is dilated by the action of prostaglandins. Glucocorticoid hormones inhibit the biosynthesis of prostaglandins and, through this action, cause closure of the ductus arteriosus. It appears likely that the umbilical artery is also maintained by the action of prostaglandins. For this reason in the present study, indomethacin, an artificially synthesized glucocorticoid hormone, was administered to pregnant rats on day 21 of gestation. As a result, the fetal umbilical artery was slightly but significantly constricted after the treatment, supporting the foregoing hypothesis. The degree of the indomethacin-induced constriction of the umbilical artery appears to differ according to the part of the artery; in the present study, distal part (level B) was constricted, while the proximal part (level A) was not. Therefore, it is likely that the distal part of the artery is constricted more easily than the proximal part.

When examined 9 hr after indomethacin-treatment, the right umbilical artery was not constricted. This would mean that the artery had been once constricted, but then recovered.

At any rate, the reason for the spontaneous closure of the left umbilical artery in the process of prenatal development remains unknown and needs to be explored in the future.

REFERENCES

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要約

ラット胎仔における腸動脈の消長とindomethacinの影響：有崎和義・山本雅子・植田宏寛・日柳政彦・江口保道（麻布大学獣医学部解剖学第2講座）——妊娠14～21日のラットの胎仔腸動脈を、アセトン・ドライアイスで急速凍結した胎仔について調べた。各凍結胎仔の後腸を腹側から水平にナイフで剝ぎ、腸動脈断面を露出させてその内径を計測した。妊娠15日において、胎仔腸動脈は、右側のもののが左側のものよりも太かった。その後、右側は次第に太くなっていくが、左側は17日に薄くなった。妊娠21日に剖検の3時間前に、妊娠ラットにindomethacinを投与すると、胎仔の右腸動脈は有意に狭帯した。この現象の理由は不明であるが、動脈管の閉鎖様式と関連して討議した。