REVISTA ESPAÑOLA DE FISIOLOGIA, 44 (2), 131-136, 1988

# Effects of Diamine Oxidase Inhibition During Pregnancy in the Rat

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(Received on October 20, 1987)

M. P. NAVA and A. FRAILE. *Effects of Diamine Oxidase Inhibition During Pregnancy in the Rat*. Rev. esp. Fisiol., 44 (2), 131-136, 1988.

The effects of inhibiting histamine catabolism, via oxidative deamination, on the course of pregnancy on rats and on their offspring were studied. Treatment with aminoguanidine, a potent inhibitor of diamine oxidase (EC 1.4.3.6), was performed during pregnancy, before histamine levels were spontaneously increased. Twenty-one day old fetuses from treated rats showed head, lung and liver hematomas with significant differences. Abnormalities of ossification were also recorded in bones of the cranial cavity, with different statistical significances. The results of the present experiment confirm that oxidative deamination is the main catabolic pathway for histamine in the rat. Organic and skeletal abnormalities found also suggest that diamine oxidase protects fetuses from histamine excesses attained during pregnancy.

Key words: Histamine, Aminoguanidine, Pregnancy.

Histamine is a biogenic amine with a wide implication in physiological and pathological processes (2, 10).

In mammalian species the principal routes involved in the catabolism of histamine are methylation (histamine N-methyl transferase, EC 2.1.1.8) and oxidative deamination (histaminase or diamine oxidase, EC 1.4.3.6) (DAO) (3, 14, 15, 19). Their corresponding catabolites (methylhistamine, methylimidazolacetic acid from methylation; imidazolacetic acid riboside and imidazolacetic acid from oxidation) are found in the urine excreted by females after the injection of radioactive histamine (12).

There is evidence that the metabolism or histamine in rats differs according to sex. After administration of $^{14}$C-histamine females eliminate histamine in its free form, while males excrete metabolized histamine (16).

Investigations by various workers indicate that DAO is strongly inhibited *in vivo* and *in vitro* by aminoguanidine (AMG). Under this inhibition female rats excrete large amounts of histamine in its free form, indicating that oxidative deami-

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nation is the main catabolic pathway for histamine in rats (5, 8, 9).

An elevated histamine forming capacity is displayed by tissues undergoing rapid growth, such as the fetus (specially the liver), wounds and certain tumors (9). Rat fetuses synthesize large amounts of histamine in the course of pregnancy, starting on day 15 until one or two days before delivery. There is also a high production of DAO by the uterus and placenta during this last third of pregnancy (2, 10, 11).

This work is therefore an attempt to study the consequences of an inhibition of histamine catabolism by means of AMG administration, during a specific period in the course of a rat's pregnancy. Days of treatment (12 to 15 of pregnancy) are included in the period of cartilage formation, but they are previous to bone appearance. Therefore, our period of AMG treatment is also included in the embryonic stage during which organogenesis occurs. It is prior to the last third of gestation when histamine levels increase spontaneously.

## Material and Methods

Female Wistar rats, weighing 250-300 g and housed in group cages with food and water *ad libitum*, were used in the present experiment. Two female rats were mated with one male counting the morning on which spermatozoa were found in vaginal smears as day 1 of pregnancy.

Pregnant rats were divided into two groups: A control group (C) that received physiological saline (i.p. injections) from days 12 to 15 of pregnancy, and an experimental group (E) treated with aminoguanidine sulfate (AMG) (Sigma) (20 mg/kg i.p. injections) during the same period as the control group.

The dose of AMG employed (20 mg/kg body weight) produces a block of DAO in all tissues and is commonly used in experiments on rats to achieve a complete inhibition of histamine catabolism (9).

All rats were killed on the 21st day of pregnancy and opened in order to examine their uteri. Reabsorption and implantation sites were determined while viable and dead fetuses were removed, counted and weighed. They were also examined for internal and external corporal hematomas, according to Wilson's procedure (23).

The study of ossified and cartilagenous fetal skeletons was carried out using the methods of DAWSON (4), SIMMONS and VAN HORN (21) and OJEDA *et al.* (17). These procedures provided good visualization of bone surfaces (Alizarin red S) and cartilagenous (Alcian blue 8GX) elements. Abnormalities appeared as an inhibition or a delay of ossification showed by a lack of staining or by abnormally wide joints between the cranial bones.

The statistical significance between the means of control and experimental groups for each parameter analyzed was evaluated by the Student's «t» test at the 5 % level of significance ($p \leq 0.05$).

## Results

*Perinatal study (table I).* — Experimental animals showed a similar number of reabsorption sites and a decrease in the number of implantations with respect to the control group. Differences were not considered statistically significant. Mean weights of fetuses recorded at the 21st day of pregnancy as well as the number of living fetuses were not statistically significant.

*Organic abnormalities (table II).* — The effects of AMG on the offspring of experimental mothers showed a large number of fetuses with head, lung and liver hematomas. All differences were sig-

Table I. *Perinatal data after administration of aminoguanidine (20 mg/kg body weight) from days 12 to 15 of rat pregnancy.*
Results are expressed as Mean ± SEM. Number of pregnant animals in parentheses. n.s. No significant.

| | Saline (20) | Aminoguanidine (30) |
|---|---|---|
| Number of implantation sites | 12.2 ± 0.28 | 11.0 ± 0.36 n.s. |
| Number of reabsorption sites | 0.6 ± 0.11 | 0.6 ± 0.10 n.s. |
| Number of living fetuses | 11.6 ± 0.32 | 10.4 ± 0.40 n.s. |
| Number of dead fetuses | — | — |
| Weights of fetuses at birth (g) | 5.1 ± 0.38 | 4.6 ± 0.10 n.s. |

nificant ($P \leq 0.01$) from corresponding control values.

*Skeletal abnormalities (table III).* — All cranial cavity bones showed an abnormal ossification process, specially for parietal bones ($p \leq 0.01$) when they were compared to the control group. Frontal bones and occipital cartilages had no significant differences.

## Discussion

Since diamine oxidase became known as one of the enzymes involved in histamine catabolism, many studies have pointed out that the uterus, placenta and plasma of pregnant rats exhibit an elevated histaminase activity (10, 13). The physiological significance of histaminase is a matter still unsolved, although a protective function of fetuses from histamine has been proposed. However, for some authors the inhibition of DAO by administration of AMG has no effects on the course of pregnancy and on the normal development of fetuses (11, 22).

These results do not agree with those studies, as we concur that the process of pregnancy is normal. However, in the present study the fetuses show organic and skeletal abnormalities manifested as corporal hematomas and ossification abnormalities in bones of the cranial cavity, these data being, therefore, in accordance with Roberts' results (18), which demonstrated alterations on fetuses and mothers after AMG treatment.

Alterations found in fetuses might be due to maintaining histamine levels above the normal values during a certain

Table II. *Corporal hematomas found in 21 day old rat fetuses whose mothers received aminoguanidine (20 mg/kg body weight) from days 12 to 15 of pregnancy.*
Results are expressed as Mean ± SEM. Number of living fetuses observed in parentheses. ** $p < 0.01$.

| Corporal hematomas | Saline (122) | Aminoguanidine (155) |
|---|---|---|
| Head | 0.2 ± 0.13 | 2.6 ± 0.25** |
| Lung | 0.4 ± 0.27 | 2.5 ± 0.32** |
| Liver | 0.4 ± 0.27 | 1.8 ± 0.26** |

Table III. *Skeletal abnormalities found in bones of the cranial cavity of fetuses.*
Pregnant rats received aminoguanidine (20 mg/kg body weight) from days 12 to 15 of pregnancy.
Results are expressed as Mean ± SEM. Number of living fetuses observed in parentheses.

| Bones | Saline (110) | Aminoguanidine (159) |
|---|---|---|
| Frontal | 0.4 ± 0.26 | 0.9 ± 0.18 n.s. |
| Parietal | 0.4 ± 0.26 | 2.2 ± 0.31** |
| Interparietal | 0.5 ± 0.34 | 1.2 ± 0.22* |
| Occipital | 0.4 ± 0.22 | 1.0 ± 0.18* |
| Occipital (cartilage) | — | 0.2 ± 0.10 n.s. |

* $p < 0.05$ ** $p < 0.01$. n.s. No significant.

period of pregnancy in the rat, as the main catabolic pathway (oxidative deamination) remains inhibited by AMG. Such a physiological state is maintained from days 12 to 15 of pregnancy. The formation of precartilaginous condensations (day 13.5) is well known to occur during this period as well as the deposition of cartilage in some regions (day 14). A process of active chondrification, with the appearance of several ossification nuclei, starts on days 15 or 16 of the rat's pregnancy (24).

Those high histamine levels could strongly modify the important equilibrium played by histamine on microcirculation (20).

Similarly the smooth muscle wall of blood vessels inactivates circulating histamine by means of DAO in the rat, resulting in the formation of imidazolacetic acid (6). Since AMG inhibits histamine catabolism, fetal corporal hematomas found in our work would suggest that some circulatory disturbances are present and that they are caused by an altered vascular histamine metabolism.

Blood flow also plays an important role in regulating the ossification process. Present results showing a lack or a delay of ossification in cranial bones may represent an alteration of the role played by histamine in microcirculation. Several reports support the idea that histamine contributes to regulate vascular ressistance to blood flow (7). It is likely that bone abnormalities observed in fetuses may be related to the block of DAO by AMG and therefore to an inhibitory process of histamine catabolism.

However, ossification is a complex process regulated by many factors. One of them may be directly affected by AMG or may be indirectly modified by histamine through the inhibition of DAO by AMG.

A toxic effect of increased levels of histamine, indirectly caused by AMG, may also be suggested from our results. High unmetabolized histamine levels would be harmful for fetuses in a pregnancy period in which DAO remains inhibited by AMG. Further support for this idea comes from findings of HOLCSLAW *et al.* (6) about histamine uptake and metabolism in the rat's vascular smooth muscle. As it has been mentioned before, this capacity to catabolize histamine forming imidazolacetic acid is very important in order to remove from circulation all the released histamine. Therefore, present findings support the hypothesis that DAO has a protective function during pregnancy.

Another possibility in favour of a protective role for DAO has also been proposed by BARDSLEY's *et al.* They conclude that monoamine oxidase (EC 1.4.3.4) as well as DAO may protect the fetoplacental unit from excesses of biogenic amines. The results of the present work perfectly agree with those of BARDSLEY *et al.* as hematomas and ossification abnormalities show the harmful effects that some biogenic amines (histamine in this case) may produce on the offspring when their concentrations are higher than those attained for a certain period of pregnancy.

In conclusion, results here presented support the fact that oxidative deamination is the main catabolic pathway for histamine in the rat as well as in its vascular tissues. Therefore, the inhibition of histamine catabolism by AMG has negative effects on processes in which blood supply is very important. Moreover, present observations evidence the important role that histamine plays in controlling microcirculation in rats.

These findings further support a physiological function of DAO to protect fetuses from excesses of histamine.

## Resumen

Se estudian los efectos que la inhibición del catabolismo de la histamina tiene, vía desaminación

oxidativa, sobre el desarrollo de la gestación en ratas, así como sobre su descendencia. El tratamiento con aminoguanidina, potente inhibidor de la diaminoxidasa (EC 1.4.3.6) se realiza durante la gestación, antes de que los niveles de histamina se eleven espontáneamente. Los fetos de 21 días, descendientes de ratas tratadas, presentan hematomas en la cabeza, pulmón e hígado, con diferencias significativas. También se registran anormalidades en la osificación de los huesos craneales con diferente significación estadística. Los resultados apoyan la hipótesis de que la desaminación oxidativa es la principal ruta catabólica de la histamina en la rata y que la diaminoxidasa protege a los fetos de las elevadas cantidades de histamina que se alcanzan durante la gestación.

Palabras clave: Histamina, Aminoguanidina, Gestación.

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