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Metabolism of Histamine During Pregnancy in the Rat

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In order to clarify histamine metabolism during the period of pregnancy in which this amine attains the highest levels, pregnant and non-pregnant rats were submitted to four different treatments: ^{14}C-histamine; ^{14}C-histamine + histamine dihydrochloride; ^{14}C-histamine + aminoguanidine; ^{14}C-histidine. Paper chromatography and autoradiographic techniques were used to separate and to detect ^{14}C-histamine metabolites. Total radioactivity excreted and percentages of histamine metabolites were calculated by liquid scintillation. Our results support two principal hypotheses: 1) Oxidation is the main catabolic pathway for histamine degradation in pregnant and non-pregnant rats. 2) Pregnant rats, in opposition to non-pregnant, show an increased capacity to metabolize histamine by oxidative deamination.

Key words: Histamine metabolism, Pregnancy.

Histamine is a biogenic amine with different physiological effects on smooth muscle contraction, neurotransmission, microcirculation, vascular permeability, immunity and gastric acid secretion (12). Its four different metabolic pathways have been widely studied in rats, mice, guinea pigs (17) and men (5), rendering more than eighteen compounds. However, there are two principal pathways in which methylation (histamine-N-methyltransferase, EC 2.1.1.8) produces methylhistamine (1-methyl-4-[β-aminoethyl]-imidazole) (MeH) and subsequently (monoamine oxidase, EC 1.4.3.4.) methylimidazolacetic acid (1-methylimidazole-4-acetic acid) (MeImAA). In the other route H is deaminated (diamine oxidase, EC 1.4.3.6.) (DAO) to form imidazolacetic acid (ImAA), which can be conjugated and excreted as imidazolacetic acid riboside (1-imidazole-4-acetic acid riboside) (12, 16). The relative importance of the pathways is different according to species. In this manner, while methylation of the ring nitrogen is the most important catabolic route in some mammals (mice, guinea pigs, cats, dogs and humans), deamination of histamine is the main catabolic route in the rat (13). There is also evidence of an important sexual difference in the catabolism of histamine in rats, as GUSTAFSSON *et al.* (6) stated in 1957. NETTER *et al.* (14) did not observe

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a distinct sexual distribution or activity of DAO, although WESTLING and WETTERQVIST (20) recorded that the male rat methylates H more effeciently than the female rat. They founded that testosterone increased the rate of methylation.

Female rats excrete a high percentage of unmetabolized endogenous H after administration of this amine (14, 22). However, the relative importance of its different catabolic pathways is not clear in the female rat. On the one hand several non-isotopic and isotopic experiments (^{14}C-L-histidine) (17, 21), which analyze the urine of female rats, support the idea that methylation is the most important metabolic route. On the other hand, a large number of investigations indicate that oxidative deamination is the predominant pathway for H to be catabolized in female rats (3, 8, 9). After ^{14}C-histamine administration, WESTLING and WETTERQVIST (20) observed in female rats that a high proportion of the dose is excreted as ImAA.

Several methods allow quantitative and qualitative determinations of histamine and its different metabolites in biological fluids (plasma, cerebrospinal liquid and urine). Fluorometric techniques (19), high-performance liquid chromatography (23) and gas chromatography (13) are the most sensitive. The evaluation of histamine and its metabolites in urine collected after radioactive histidine (2) or histamine injection has also been reported. Rat urine measurements over a certain period of time give reliable information about the mean histamine production or liberation rate during this time (21).

In order to clarify histamine catabolism and since histamine levels greatly increase between days 15 to 19 of rat pregnancy, this report studies histamine excretion patterns after ^{14}C-histamine and ^{14}C-histidine administration in pregnant rats. The consequences of aminoguanidine (AMG) administration, which inactivates oxidation of histamine to ImAA (DAO or histaminase) (9) providing information about unchanged histamine in the urine, and histamine dihydrochloride administration are also studied during pregnancy. Concerning to non-radioactive histamine, the amount injected is similar to that synthetized by the fetuses in a pregnant rat. The purpose of this administration is to ascertain the saturation level and order of histamine catabolyc enzymes according to the metabolites recorded.

Materials and Methods

Products. — Aminoguanidine (Sigma) and Histamine dihydrochloride (Calbiochem). Isotopic compounds: ^{14}C-L-histidine (59 mCi/mmol) and ^{14}C-histamine dihydrochloride (56 mCi/mmol), both ring 2-^{14}C (Amersham International).

Animals and procedure. — Female Sprague-Dawley rats weighing 200-300 g were divided into three groups of five animals per group: (I) non-pregnant animals; (II) pregnant animals with 15 days of pregnancy, and (III) pregnant animals with 20 days of pregnancy.

These days were chosen according to the different histamine levels that a rat attains during pregnancy.

Rats were not fed for 18 h after which each group received i.p. injections of the following treatments: 5 μCi ^{14}C-histamine; 5 μCi ^{14}C-histamine + histamine dihydrochloride (8 mg/kg body weight); 5 μCi ^{14}C-histamine + aminoguanidine (20 mg/kg body weight), and 5 μCi ^{14}C-histidine.

Pregnant rats received their treatments on days 15 and 20 of pregnancy. After each corresponding treatment, rats were housed in metabolic cages and urine was continuously collected over two periods of 0-8 h and 8-24 h. A drop of HCl was added to the collection bottles in order to maintain a low pH level.

Urine samples were measured for total

radioactivity in an Intertechnique SL 30 liquid scintillation system. Histamine and histamine metabolites were separated by paper chromatography (21 × 5 cm Whatman paper No. 4 strips), according to SCHWARTZMAN's (18) procedure. In order to identify accurately the spots corresponding to histamine and its metabolites, two different solvent systems were used. Although the first one (n-butanol-acetic acid-water, 60:22:23) (18) yielded a best resolutive power, the second one (ethanol-ammonia-water, 70:5:25) (1) allowed us to compare results obtained with both solvents. Localization of metabolites was carried out by using non radioactive specific standards (Calbiochem) corresponding to the metabolites studied in this work. Autoradiographic techniques were used to detect ^{14}C-metabolites and the ^{14}C in each metabolite was counted by liquid scintillation. Total radioactivity excreted, as well as the corresponding percentages for histamine metabolites, were also calculated according to total ^{14}C administered in each period.

Results were expressed as percentages (mean ± SEM) of total ^{14}C collected in each period. Those compounds not represented in figures indicated that although they were measured, their corresponding values were 0.

Each metabolite was compared with the same metabolite in the other groups, under the same period of time and treatment. Statistical comparisons were evaluated by the analysis of variance. Mean differences were tested by the Duncan's New Multiple Range Test, being $p < 0.05$ the criterion for statistical significance.

Results

The results for pregnant and non-pregnant rats (figure 1) indicate that ImAA was the main metabolite excreted in both periods of urine collection, although histamine, MeH and MeImAA peaks also

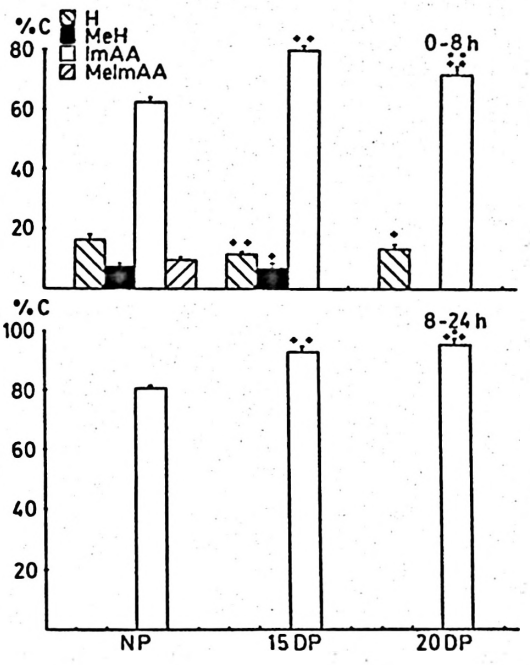

Fig. 1. *Elimination rates for histamine (H) and histamine metabolites (methylhistamine, MeH; imidazolacetic acid, ImAA; methylimidazolacetic acid, MeImAA) after ^{14}C-histamine administration.* Results are expressed as mean percentages (mean ± SEM) of total ^{14}C collected in each period. NP = Non-pregnant; 15 DP = 15 days pregnant; 20 DP = 20 days pregnant. + $p \leq 0.05$ ++ 0.01, vs NP; • 0.05 •• $p \leq 0.01$, vs 15 DP.

appeared in the first 8 h after the injection of ^{14}C-histamine. MeImAA was not detected in chromatograms of pregnant rats. This data indicates that the major catabolic pathway under radioactive histamine administration was via formation of ImAA by DAO in the first 24 h of excretion.

Non-pregnant females eliminated most of the ^{14}C-injected as unmetabolized histamine during the first period, while pregnant rats excreted ImAA as the major metabolite followed by MeImAA (figure 2). On the contrary, the second period (8-24 h) showed for all groups a normal histamine elimination pattern. ImAA was

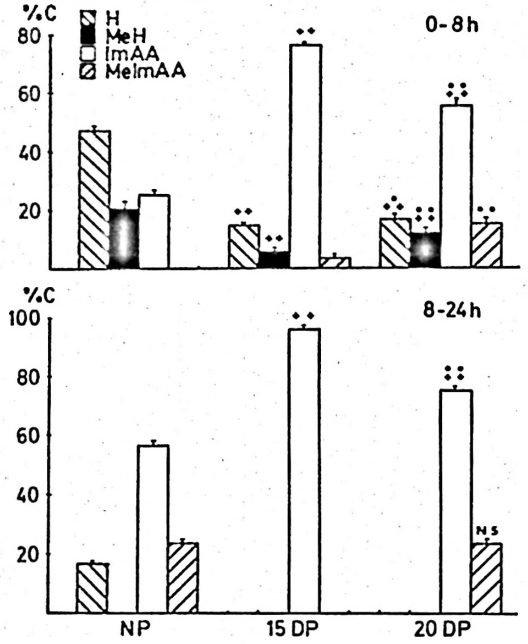

Fig. 2. *Elimination rates for histamine (H) and histamine metabolites (methylhistamine, MeH; imidazolecetic acid, ImAA; methylimidazolacetic acid, MeImAA) after ^{14}C-histamine + histamine dihydrochloride administration.*

Results are expressed as mean percentages (mean ± SEM) of total ^{14}C collected in each period. NP = Non-pregnant; 15 DP = 15 days pregnant; 20 DP = 20 days pregnant. ++ $p \leq 0.01$, vs NP; • $p \leq 0.05$ •• $p \leq 0.01$, vs 15 DP; ns = not.

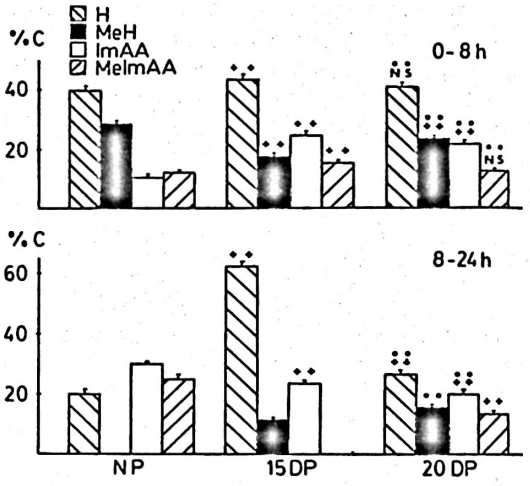

Fig. 3. *Elimination rates for histamine (H) and histamine metabolites (methylhistamine, MeH; imidazolacetic acid, ImAA; methylimidazolacetic acid, MeImAA) after ^{14}C-histamine + aminoguanidine administration.*

Results are expressed as mean percentages (mean ± SEM) of total ^{14}C collected in each period. NP = Non-pregnant; 15 DP = 15 days pregnant; 20 DP = 20 days pregnant. ++ $p \leq 0.01$, vs NP; •• $p \leq 0.01$, vs 15 DP; ns = not significant.

the main metabolite excreted. Because the pregnant female was still eliminating ImAA and the non-pregnant female had already metabolized the overdose of histamine to ImAA. MeImAA also appeared in chromatograms of non-pregnant and 20 day pregnant rats.

As was expected, administration of AMG, which inhibits DAO, caused similar results in the first period (0-8 h) in non-pregnant and pregnant animals (fig. 3). Both groups showed a substancial increase in urinary unchanged histamine, MeH and MeImAA, whereas the percentage of ImAA was low compared to histamine elimination.

In the second period (8-24 h), non-pregnant rats displayed a normal excretion pattern. Females were still eliminating histamine but ImAA and MeImAA were the major metabolites. However, differences were found in this period between both groups of pregnant rats. While females 15 days pregnant eliminated H in the highest percentage, females 20 days pregnant excreted more ImAA than any other product.

Under this treatment chromatograms of pregnant and non-pregnant rats showed two principal peaks in both periods (figure 4). All groups eliminated during the first period (0-8 h) more ^{14}C-histamine (formed by decarboxylation of

^{14}C-histidine injected) than any other metabolite. Nevertheless, the second period pointed out some differences because non-pregnant rats eliminated ^{14}C-histamine at the highest percentage, while pregnant rats excreted more ImAA than ^{14}C-histamine.

^{14}C elimination rates for all groups under the four different treatments were summarized in table I.

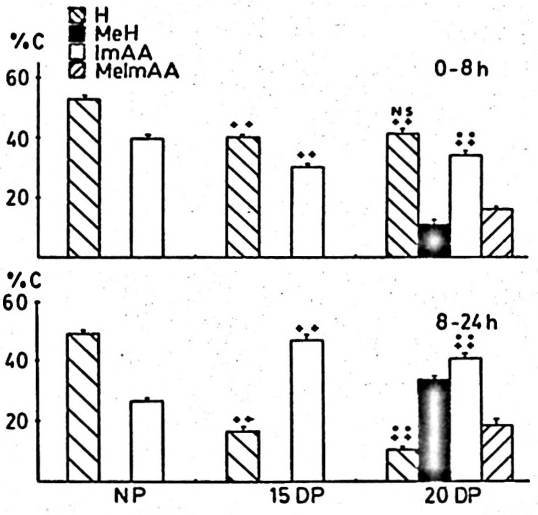

Fig. 4. *Elimination rates for histamine (H) and histamine metabolites (methylhistamine, MeH; imidazolacetic acid, ImAA; methylimidazolacetic acid, MeImAA) after ^{14}C-histidine administration.* Results are expressed as mean percentages (mean ± SEM) of total ^{14}C collected in each period. NP = Non-pregnant; 15 DP = 15 days pregnant; 20 DP = 20 days pregnant. ++ $p \leq 0.01$, vs NP; •• $p \leq 0.01$, vs 15 DP; ns = not significant.

Discussion

Investigations on histamine elimination patterns during pregnancy were initiated many years ago when SCHAYER (15) injected ^{14}C-histamine to pregnant and non-pregnant women. Later, LINDBERG *et al.* (11) observed that in contrast to non-pregnant women, in which MeH and MeImAA are the major ^{14}C-histamine metabolites, pregnant women show a high rate of ImAA excretion.

According to the results obtained under ^{14}C-histamine administration, ImAA is excreted at high percentages in the first and the second periods. The observations for pregnant rats agree with those of FRAILE and PUERTA (4) in non-pregnant rats, indicating that most of the ^{14}C injected is eliminated as ImAA during the first 8 h of urinary collection. In agreement with these investigations our findings support the fact that oxidation is the main catabolic pathway for histamine in pregnant rats.

Table I. *^{14}C elimination rates in urine expressed as percentages of total ^{14}C administered (Mean ± SEM).*
NP = Non-pregnant; 15 DP = 15 days pregnant; 20 DP = 20 days pregnant.

TREATMENTS	PERIODS (h)	NP	15 DP	20 DP
^{14}C-histamine	0 — 8	78.54 ± 0.40	85.27 ± 0.30	78.21 ± 0.23
	8 — 24	13.38 ± 0.61	12.26 ± 0.66	10.55 ± 0.98
^{14}C-histamine + H dihydrochloride	0 — 8	78.38 ± 0.34	91.32 ± 0.36	75.52 ± 0.24
	8 — 24	18.23 ± 0.68	7.19 ± 0.72	15.03 ± 0.48
^{14}C-histamine + aminoguanidine	0 — 8	83.56 ± 0.25	94.39 ± 0.62	82.35 ± 0.73
	8 — 24	14.07 ± 0.59	2.43 ± 1.01	4.72 ± 0.88
^{14}C-histidine	0 — 8	74.77 ± 0.58	81.85 ± 0.56	72.38 ± 0.70
	8 — 24	15.35 ± 0.73	8.24 ± 0.75	5.37 ± 0.65

As mentiond before, when histamine is injected at a very high dose ^{14}C-histamine is the principal compound eliminated by non-pregnant rats, as demonstrated by the excreted metabolites in the first period. However, pregnant rats show a high percentage of ImAA elimination, independently of the overdose of histamine administered and of the large amounts of histamine synthesized by the fetuses from the 15th day of pregnancy.

Results of the present experiment seem to indicate that pregnant rats have sufficient DAO to oxidize the overdose of histamine to ImAA more efficiently, unlike non-pregnant rats which mainly excrete unmetabolized ^{14}C-histamine. These observations of an increase in ImAA excretion in pregnant rats are in accordance with an important characteristic of rat pregnancy. An enormous increase in the histaminolytic power of the uterus and placenta occurs during pregnancy. In addition, large amounts of histamine synthesized by the fetuses do traverse the powerful histamine-destroying placental and uterine barriers. Moreover, our results demonstrate these statements, as rats have a large metabolizing capacity. They also confirm the results obtained by KAHLSON and ROSENGREN (10), who reported that ^{14}C-histamine crosses the placental barrier in a metabolized form. In addition; findings of the present experiment also show that oxidation is the principal metabolic route in pregnant rats.

As mentioned before, our results show that histamine oxidation is almost completely inhibited by AMG in all groups. Furthermore, the decrease in the synthesis of ImAA by AMG treatment is partly compensated by an elevated formation of MeH and MeImAA. There is also an increase in the fraction excreted as unchanged histamine. The present observation during the pregnancy period confirm several works (3, 14), which state that oxidative deamination by DAO is the major catabolic pathway in the rat, since blockade of DAO by AMG gives to an inhibition of histamine catabolism.

Regarding the different percentages of oxidation observed in rats 15 and 20 days pregnant in the second period (8-24 h), results support the previous theory of a higher enzymatic capacity in pregnant rats. Since histamine synthesized by the fetuses increases from the 15th day of pregnancy until one or two days before delivery, it would follow that higher histamine levels would correspond with increasing amounts of histamine catabolic enzymes (DAO in this case) and therefore with increasing amounts of ImAA. However, oxidative deanimation remains inhibited by AMG, what would explain the lower urinary ImAA levels found in rats 15 and 20 days pregnant.

According to WETTERQVIST and WHITE (21) the injected radiolabelled histidine may follow two different pathways. In the first one ^{14}C-histamine is rapidly formed from ^{14}C-histidine, but it is not stored or it is stored for a short time. It is metabolized at a high rate and mainly excreted as histamine in the first 24 h. In the second pathway histidine is stored or is slowly decarboxylated to histamine to be subsequently metabolized.

The results for the first period (0-8 h) indicate that ^{14}C-histidine is metabolized to histamine and partly deaminated to ImAA in pregnant and non-pregnant rats. However, the differences recorded during the second period (8-24 h) of urine collection indicate a larger enzymatic oxidation capacity of pregnant rats. In fact, while non-pregnant animals are not able to metabolize all the synthesized ^{14}C-histamine to ImAA, pregnant rats normally do metabolize ^{14}C-histidine to ^{14}C-histamine and afterwards to ImAA.

In conclusion, results of this study confirm the previously exposed hypothesis about an increased enzymatic capacity of pregnant rats to catabolize histamine, independently of its circulating levels.

This catabolism follows the oxidative deaminating pathway via DAO. Obviously, more studies are necessary for a better understanding of histamine metabolism throughout pregnancy.

Resumen

Se estudia el metabolismo de la histamina durante la etapa gestacional en la que alcanza sus valores más altos en ratas gestantes y no gestantes sometidas a cuatro tratamientos diferentes: ^{14}C-histamina; ^{14}C-histamina + clorhidrato de histamina; ^{14}C-histamina + aminoguanidina; ^{14}C-histidina. La separación y detección de los metabolitos de la histamina se realiza mediante cromatografía en papel y técnicas autorradiográficas. La radiactividad total excretada y los porcentajes correspondientes a los metabolitos se miden mediante centelleo líquido. Los resultados apoyan dos hipótesis importantes: La oxidación es la principal ruta catabólica que degrada la histamina en ratas gestantes y no gestantes, las ratas gestantes, contrariamente a las no gestantes, presentan mayor capacidad para metabolizar la histamina mediante desaminación oxidativa.

Palabras clave: Metabolismo de la histamina, Gestación.

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