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Effects of pregnancy on tryptophan metabolism and disposition in the rat

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Tryptophan availability in rat serum is increased throughout pregnancy, because of inhibition of liver tryptophan pyrrolase activity during the first 15 days and decreased protein binding thereafter.

INTRODUCTION

As an essential amino acid, tryptophan is vital for protein synthesis and may thus play an important role in cellular differentiation, development and growth. Evidence from teratological [1], developmental [2] and biochemical [3] studies is consistent with these possibilities. However, despite this apparent importance of tryptophan, surprisingly little is known about its metabolism and disposition during pregnancy. Thus the activity of liver tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11), which is a major determinant of tryptophan availability (for review, see [4]) has been reported to be enhanced on days 13 [5] and 19–20 [6] of pregnancy in rats. An enhancement in late pregnancy preceded by an earlier inhibition have also been reported, though in abstract form [7,8]. This pregnancy-induced enhancement does not appear at first surprising, because of the widely held view that oestrogens may be responsible. This view has been accepted ever since it was demonstrated [9] that chronic oestradiol administration enhances pyrrolase activity in non-pregnant rats, a finding confirmed subsequently by some [10–12], but not others [5]. More recently evidence for an inhibitory effect of oestrogens on hepatic tryptophan pyrrolase activity and tryptophan metabolism in rat and man has been reported [13–15]. In view of these conflicting findings and the inadequate knowledge of tryptophan metabolism and disposition in pregnancy, a detailed assessment was made in rats of both liver tryptophan pyrrolase activity and circulating tryptophan availability and also of cerebral 5-hydroxytryptamine synthesis in relation to mood changes in pregnancy and oestrogen therapy.

MATERIALS AND METHODS

Locally bred female Wistar rats (about 200 g at the start of experiments) were maintained *ad libitum* on cube diet 41B (Oxoid) and water, and were killed between 12:00 and 14:00 h either by stunning and cervical dislocation (for the determination of liver tryptophan pyrrolase activity) or by decapitation (for all other determinations). Pregnancy was established by the appearance of a vaginal plug, and pregnant rats were housed four per cage (under natural light/dark cycles and at 22±1 °C) for various periods as indicated.

Chemicals were purchased from BDH Chemicals and Sigma (both of Poole, Dorset, U.K.) and were of the purest commercially available grades. Oestradiol or progesterone (5 mg/kg body wt. each) was dissolved in dimethylformamide and was administered intraperitoneally to non-pregnant rats. Control animals received an equal volume (1 ml/kg) of the solvent.

Tryptophan pyrrolase activity was determined in fresh liver homogenates [16,17] either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added (2 μM) haematin. The apoenzyme activity was obtained by difference. Concentrations of free (ultrafiltrable) serum, total (acid-soluble) serum and brain tryptophan, brain 5-hydroxytryptamine and its major metabolite 5-hydroxyindol-3-ylacetic acid and serum albumin and non-esterified fatty acids were all determined by standard procedures (for references, see [18]). Results were analysed statistically by Student's t test.

RESULTS AND DISCUSSION

Effects of pregnancy on rat liver tryptophan pyrrolase activity

As shown in Fig. 1, liver tryptophan pyrrolase activity was decreased as early as 1 day after the onset of pregnancy. Thus, on day 1, the holoenzyme and total enzyme activities were decreased by 37% and 49% respectively ($P < 0.001$); the apoenzyme activity (obtained by difference) was therefore decreased at this time interval by 58% ($P < 0.001$). This pyrrolase inhibition was maintained with the progress of pregnancy, reaching a maximum (47%, 66% and 80% for the holoenzyme, total enzyme and apoenzyme respectively; $P < 0.001$) on day 9. Pyrrolase activities then began to recover gradually, but were still significantly decreased on day 15 (by 20%, 16% and 13% respectively; $P = 0.05$–0.001). Complete recovery was finally reached on day 16, and no further significant changes occurred during the remaining 4–5 days of pregnancy, nor during the subsequent 7 days after parturition. These detailed results of liver tryptophan pyrrolase activity during pregnancy in rats provide no evidence for any enhancement of the enzyme activity, in contrast with previous reports (see the Introduction). No explanation of these differences in results can as yet be offered.

The decrease in pyrrolase activity could be caused by: (*a*) inhibition of apoenzyme synthesis; (*b*) inactivation of the apoenzyme, by prevention of its conjugation with haem or by an allosteric mechanism; (*c*) inhibition of the holoenzyme activity through decreased haem availability or interference with enzyme-bound haem. Addition of an excess of haematin to liver homogenates from 9-day-pregnant rats reversed the decrease in the total pyrrolase

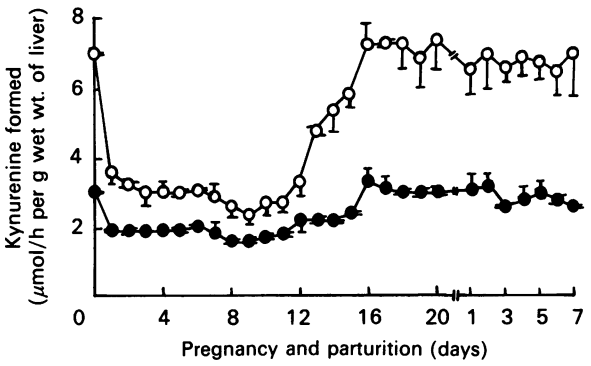

Fig. 1. Rat liver tryptophan pyrrolase activity during pregnancy and parturition

Experimental details are as described in the Materials and methods section. Liver tryptophan pyrrolase activity was determined either in the absence (holoenzyme activity; ●) or in the presence (total enzyme activity; ○) of added (2 μM) haematin. The values obtained during pregnancy and after parturition are means ± S.E.M. for each group of four rats, whereas the control values (indicated as zero time) are means of 80 rats tested in groups of four each on 20 out of the 27 experimental test days. Among these control groups, there were no significant differences in either holoenzyme or total enzyme activity between the highest (3.2 ± 0.23 and 7.4 ± 0.81) and lowest (2.7 ± 0.11 and 6.3 ± 0.47) values.

activity. Thus pyrrolase activities in the absence (i.e. holoenzyme) and the presence of 2 μM- (i.e. total enzyme) or of 8 μM- (excess of) haematin (in μmol of kynurenine formed/h per g wet wt. of liver, ± S.E.M. for each group of three rats) were as follows: control rats (2.8 ± 0.03, 6.2 ± 0.03 and 6.0 ± 0.20 respectively); 9-day-pregnant rats (1.9 ± 0.10, 3.4 ± 0.12 and 6.6 ± 0.03 respectively). These results therefore exclude inhibition of enzyme synthesis, but support prevention of the conjugation of the apoenzyme with haem, as a mechanism of the effect of pregnancy on liver tryptophan pyrrolase activity. Progesterone inhibits pyrrolase activity by this latter mechanism [10], and its circulating concentrations are known (see [19] and references cited therein) to be elevated very early in pregnancy in rats. This hormone may therefore be involved in the pyrrolase-inhibitory effect of pregnancy observed in the present work.

Further support for this possibility is provided by the finding that, as in untreated pregnant rats, both the holoenzyme and total pyrrolase activities were significantly decreased at 2 h after progesterone (5 mg/kg) administration to non-pregnant rats, with full expression of the apoenzyme being restored by the addition *in vitro* of an excess of haematin. Thus pyrrolase activities (expressed as above; means ± S.E.M. for each group of four rats) in the absence or the presence of 2 μM- and 8 μM-haematin were as follows: control (dimethylformamide-treated) rats (2.7 ± 0.15, 6.2 ± 0.47 and 6.0 ± 0.59 respectively); progesterone-treated rats (1.8 ± 0.06, 4.0 ± 0.25 and 5.8 ± 0.31 respectively). Under similar conditions, administration of oestradiol (5 mg/kg) 2 h previously also exerted similar effects (2.0 ± 0.08, 4.1 ± 0.19 and 5.6 ± 0.29 respectively). We have also found (C. J. Morgan, N. R. Davis & A. A.-B. Badawy, unpublished work) that similar effects occur in starved male rats after acute administration of synthetic oestrogens, which are known [20] to decrease liver haem by enhancing its destruction. Oestrogen concentrations are also elevated in pregnancy [19], and together with those of progesterone begin to decline around the time of the pyrrolase recovery. Taken together, this suggests that pregnancy inhibits rat liver tryptophan pyrrolase activity through both progestogens and oestrogens by two mechanisms: prevention of conjugation of the apoenzyme with haem and decreased haem availability.

Effects of pregnancy on tryptophan metabolism and disposition

Liver tryptophan pyrrolase is a major peripheral determinant of tryptophan availability [4]. The results in Table 1 show that, at the time intervals of pregnancy during which pyrrolase activity was inhibited, both free serum and total serum tryptophan concentrations were significantly ($P = 0.05$–0.001) increased to approximately equal extents (23 % on day 1; 20–21 % on day 4; 25–31 % on days 8 and 12 of pregnancy). These relatively similar increases therefore were not associated with altered binding of tryptophan to serum proteins (as expressed by the percentage of free serum tryptophan), and this is consistent with the absence of any significant changes in concentrations of serum non-esterified fatty acids (the physiological displacers of albumin-bound tryptophan). Although serum albumin concentration was decreased by 8–16 % during the first 12 days of pregnancy, this was presumably insufficient to cause release of bound tryptophan *in vivo*.

Proportionate increases in free serum and total serum tryptophan concentrations (of the extents described above) in the absence of altered tryptophan binding are typical of observations related to the effects of inhibition of liver tryptophan pyrrolase activity on circulating tryptophan availability [21]. The results in Table 1 also show that, in late pregnancy (days 16 and 20), when liver pyrrolase activity was no longer inhibited, only free serum tryptophan concentration remained elevated (by 36 % on day 16 and 122 % on day 20). By contrast, total serum tryptophan concentration returned to near-normal values on day 16 and was decreased by 21 % on day 20. As a result of decreased tryptophan binding to serum proteins in late pregnancy, the percentage free serum tryptophan increased by 33 % on day 16 and a surprisingly large 180 % on day 20. At these times, serum albumin concentration was decreased by 19 % and 26 % on days 16 and 20 respectively, whereas non-esterified fatty acid concentration was elevated only on day 20, by 76 %. It may therefore be suggested that a decrease in serum albumin concentration *in vivo* of 19 % or more can cause a significant decrease in tryptophan binding to serum proteins, as happens on day 16 of pregnancy, and that the greater decrease in this binding on day 20 may be the combined result of decreased albumin, and more importantly, increased non-esterified fatty acid, concentration. This latter increase may be secondary to enhanced lipolysis in later pregnancy [22]. Sex hormones are unlikely to be involved as direct displacers of protein-bound tryptophan in later pregnancy, because their concentrations then decline, rather than rise [19]. Similarly, any likely involvement of elevated serum corticosterone concentrations just before parturition [19] can be excluded, because this steroid does not influence serum tryptophan binding [23].

The results in Table 1 also show that the increase in

Table 1. Effects of pregnancy on rat tryptophan metabolism and disposition and on concentrations of serum albumin and non-esterified fatty acid

Experimental details are as described in the Materials and methods section. Serum tryptophan, albumin and non-esterified fatty acid concentrations are in μg/ml, g/l and mM respectively, whereas brain indoles are in μg/g wet wt. Values are means ± S.E.M. for each group of six rats. The results obtained in test rats on specific days of pregnancy are compared with those observed with those in control animals examined on the same days, and the significance of the differences is indicated as follows: † $P < 0.05$; * $P < 0.025$; ** $P < 0.005$; *** $P < 0.001$. Abbreviations used: Trp, tryptophan; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindol-3-ylacetic acid; NEFA, non-esterified fatty acids.

	Serum Trp					Brain indoles		
Pregnancy day	Free	Total	Free (%)	Serum albumin	Serum NEFA	Trp	5-HT	5-HIAA
Control	0.84±0.04	22.43±0.63	3.74±0.22	42.1±1.1	0.52±0.03	2.01±0.06	0.79±0.037	0.22±0.014
Day 1	1.03±0.07†	27.60±1.11**	3.73±0.27	44.2±0.7	0.50±0.04	2.46±0.11**	0.90±0.021†	0.27±0.021†
Control	1.25±0.04	23.05±0.39	5.42±0.22	44.3±0.4	0.41±0.02	1.75±0.04	0.71±0.004	0.32±0.006
Day 4	1.50±0.03***	28.00±1.18**	5.36±0.17	40.2±0.5***	0.44±0.04	2.25±0.06***	0.89±0.022***	0.41±0.013***
Control	1.06±0.02	25.96±0.44	4.08±0.09	42.7±0.6	0.41±0.03	1.91±0.01	0.75±0.013	0.48±0.010
Day 8	1.39±0.03***	32.42±0.87***	4.29±0.09	37.9±0.7***	0.44±0.06	2.27±0.02***	0.86±0.015***	0.55±0.014***
Control	1.05±0.01	26.24±0.22	4.00±0.04	41.4±1.9	0.52±0.01	2.20±0.03	1.08±0.022	0.39±0.014
Day 12	1.38±0.04***	32.80±0.56***	4.21±0.07	34.8±1.4*	0.51±0.06	2.88±0.06***	1.27±0.023***	0.47±0.010***
Control	1.23±0.04	27.08±0.40	4.54±0.17	44.7±0.3	0.40±0.04	2.41±0.03	0.64±0.020	0.39±0.006
Day 16	1.67±0.06***	27.70±0.98	6.03±0.21***	36.2±0.2***	0.43±0.04	2.92±0.05***	0.74±0.012**	0.47±0.007***
Control	1.14±0.03	23.30±0.22	4.89±0.20	48.0±3.5	0.38±0.06	1.96±0.04	0.65±0.008	0.41±0.004
Day 20	2.53±0.10***	18.50±0.26***	13.68±0.51***	37.9±2.4†	0.67±0.10†	2.67±0.08***	0.84±0.018***	0.62±0.018***

circulating tryptophan availability observed throughout pregnancy in rats was reflected in increases in brain tryptophan and 5-hydroxyindole concentrations of 14–23%, 25–29%, 15–19%, 18–31%, 16–21% and 36–51% on days 1, 4, 8, 12, 16 and 20 of pregnancy respectively. That the increase in brain tryptophan concentration on day 20 of pregnancy (36%) did not match that in free serum tryptophan (122%) is difficult to explain; a possible increase in hydroxytryptamine turnover, as suggested by the greater rise in 5-hydroxyindol-3-ylacetic acid concentration (51%), or a possible preferential diversion of circulating tryptophan to the fetus may be responsible.

General conclusions

The present results have demonstrated that circulating tryptophan availability is increased throughout pregnancy in rats. This may be an important physiological event in relation to fetal development and growth. We have not measured the concentration of other circulating amino acids in the present work, and cannot therefore discuss the specificity of the tryptophan changes observed, except insofar as they relate to the mechanisms discussed above. Although evidence generally points to a sparing effect of pregnancy on essential amino acids [24–26], no information is as yet available on any likely specific changes in those other than tryptophan. Another point of interest is that, because of evidence from the present and previous work [14] work that oestrogens, progesterone and pregnancy all inhibit liver tryptophan pyrrolase activity and thus increase tryptophan availability to the brain in rat and/or man, the long-held view that the incidence of depression in some oral-contraceptive users and/or during pregnancy is caused by a defective cerebral 5-hydroxytryptamine synthesis secondarily to oestrogen-mediated pyrrolase induction (see, e.g. [27]) requires fresh appraisal.

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