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DECREASED PHENYLETHANOLAMINE-*N*-METHYLTRANSFERASE AND CATECHOL-*O*-METHYLTRANSFERASE ACTIVITY IN RABBIT ADRENAL GLANDS DURING PREGNANCY

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1 Phenylethanolamine-*N*-methyltransferase (PNMT) activity in adrenal gland was lower than the control values at day 24 of rabbit pregnancy and fell throughout the gestational period.

2 Catechol-*O*-methyltransferase (COMT) activity during pregnancy and parturition followed a similar pattern to PNMT.

3 Adrenaline content of the adrenal gland declined significantly during the last few days of pregnancy but at parturition showed a tendency to return towards normal.

4 The biochemical mechanisms which result in a decline in PNMT and COMT activities and adrenaline concentration appear to be the consequence of modifications in endocrine gland secretion during pregnancy.

Introduction

The role of adrenergic neurotransmission in the maintenance of pregnancy has been discussed by many investigators (Fuchs, 1971). Animal experiments provide evidence that the monoamine content of peripheral and central nervous systems is altered during pregnancy (Kopin & Wurtman, 1963; Sjöberg, 1968). The binding, uptake and metabolism of radioactively labelled amines by the tissues of pregnant animals differ from those of non-pregnant female animals (Wurtman, Axelrod & Potter, 1964; Parvez, Parvez & Youdim, 1975). Our previous studies have shown that the processes of monoamine regulation have an important link with the termination of pregnancy (Parvez, Gripois & Parvez, 1973a; Parvez, Parvez & Gripois, 1973b) in the rabbit, whilst pregnant women have a significantly elevated neurotransmitter monoamine content in the blood close to onset of labour (Zuspan, 1970; Zuspan & Zuspan, 1973). The present study was designed to investigate the influence of pregnancy and parturition upon monoamine biosynthetic and catabolic enzymes in the rabbit adrenal gland.

Methods

Variations in rates of adrenaline biosynthesis and catabolism were measured by determining the activities of the enzymes phenylethanolamine-*N*-methyltransferase (PNMT) and catechol-*O*-methyltransferase (COMT). Adrenal monoamine content was also determined at those times during pregnancy when activities of the enzymes PNMT and COMT were likely to have changed. Albino rabbits of New Zealand strain were used. They were impregnated in the laboratory and kept in separate cages in an animal house at a constant temperature of 20°C with exposure to natural light and darkness. Under these conditions, parturition occurred during days 31–32 post coitum. At specified days of pregnancy, the animals were killed by neck fracture and the adrenals were rapidly excised.

*Assay of phenylethanolamine-*N*-methyltransferase*

The right adrenal was homogenized in 2 ml ice cold 0.9% (w/v) KCl and the homogenate centrifuged for 30 min at 50,000 *g* at 4°C. The supernatant was used as the source of the enzyme. The incubation mixture consisted of 0.2 ml phosphate buffer (0.5 M, pH 7.9), 20 µl of normetadrenaline solution (4.5 mg/ml), 0.2 ml enzyme preparation and 0.1 ml of *S*-adenosyl-methionine-[^{14}C-methyl] (20 nmol). The mixture was incubated for 1 h at 37°C. The reaction was stopped by addition of 0.5 ml borate buffer (0.5 M, pH 10). This was followed by addition of 4 ml toluene plus isoamyl alcohol (3:2 ratio). The tubes were shaken mechanically for 20 min and centrifuged for 5 min at

5000 *g*; 3 ml of the organic phase was transferred to a scintillation bottle containing 10 ml Bray's phosphor. The ezyme activity is expressed either as ct/min per whole adrenal gland or as ct min^{-1} g^{-1} of adrenal gland, since the extracted ct/min showed direct proportionality to the amount of enzyme preparation used (Parvez & Parvez, 1973).

Assay of catechol-O-methyltransferase

COMT was assayed using *S*-adenosylmethionine-[^{14}C-methyl] (Parvez & Parvez, 1973) as methyl donor. The enzyme preparation was the same as for PNMT. The incubation mixture consisted of 0.2 ml phosphate buffer (0.5 M, pH 7.9), 20 µl $MgCl_2$, 20 µl (–)-adrenaline (0.5 µM), 0.1 ml *S*-adenosylmethionine-[^{14}C-methyl] (20 nmol) and 0.2 ml enzyme preparation. The rest of the procedure was the same as that used for the assay of PNMT. The enzyme activity is expressed as described above.

Adrenal monoamine determination

The left adrenal gland was freed from fat, weighed and homogenized in 2 ml 4% (w/v) trichloroacetic acid in an all glass homogenizer. After centrifugation at 50,000 *g*, the supernatant was assayed for adrenaline by the method of Euler & Lishajko (1961): the trihydroxindole reaction was employed for spectrofluorometric determination of adrenaline.

All results are shown with standard errors of the mean. The significance of differences between means was calculated using Fisher's *t* test.

Results

Figure 1 shows variations in PNMT activity during the course of pregnancy. Activity was lower than control values at day 24 of pregnancy and was observed to fall throughout the gestational period whether the results are expressed as ct min^{-1} g^{-1} wet wt or as ct/min per adrenal. Enzyme activity, when expressed in ct min^{-1} g^{-1} wet wt, showed a constant decline to the end of parturition. At

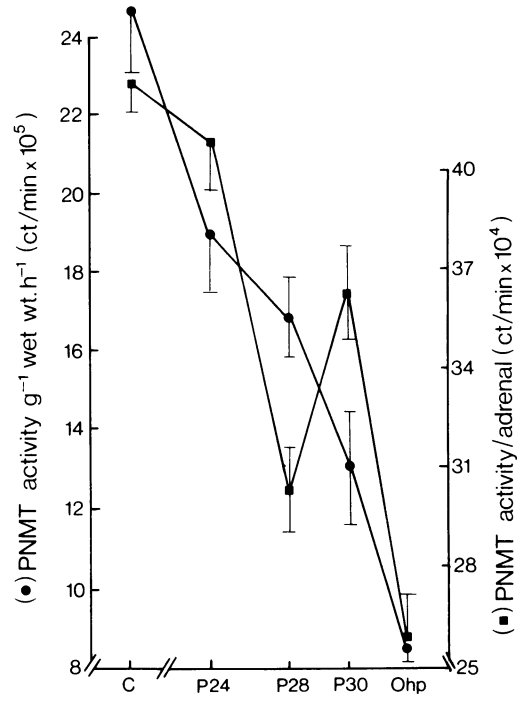

Figure 1 Activity of phenylethanolamine-*N*-methyltransferase (PNMT) in the adrenal glands of non-pregnant, (C) and pregnant rabbits (P24, 28 and 30 days post coitum) and rabbits at parturition (0hP, zero hour after birth when all the foetuses were born). The results are expressed as mean of 6 to 12 animals. Vertical lines show s.e. mean. Significance of differences between groups: C *vs* 0hP, $P<0.001$ (ct min^{-1} g^{-1} and ct min^{-1} per adrenal; 30 days post coitum *vs* 0hP, $P<0.01$ (ct min^{-1} g^{-1} and ct min^{-1} per adrenal).

parturition, PNMT activity (ct min^{-1} g^{-1}) was one third of control value. PNMT activity showed a significant increase on day 30 post coitum when compared with the value on day 28 when the results were expressed as ct/min per adrenal. COMT activity during pregnancy and parturition (Figure 2) showed a

Table 1 Adrenaline content of rabbit adrenal glands during pregnancy

Animals	*Adrenaline (µg/pair)*	*Adrenaline (µg/g wet wt.)*	*% of Control (µg/g)*	*(µg/pair)*
Control	65 ± 10	600 ± 50		
28 days post coitum	40 ± 15	410 ± 40	60%	68%
30 days post coitum	20 ± 5	320 ± 20	30%	53%
0 hour parturition	45 ± 8	410 ± 10	69%	68%

The results are expressed as mean (± s.e. mean of 10 to 15 animals). Significance of differences between groups: control vs 30 days post coitum, $P<0.005$ (µg/g), $P<0.001$ (µg/pair) and 30 days post coitum vs 0 hour parturition, $P<0.002$ (µg/g) and $P<0.002$ (µg/pair).

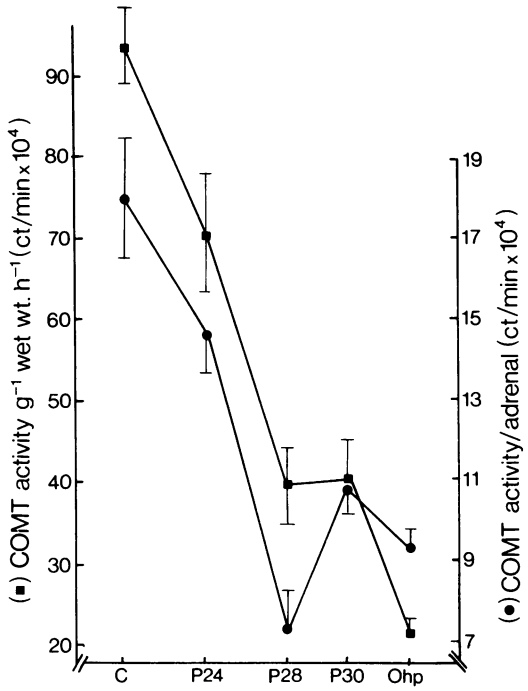

Figure 2 Activity of the enzyme catechol-*O*-methyltransferase (COMT) in the adrenal glands of non-pregnant (C) and pregnant rabbits (P24, 28 and 30 days post coitum) and rabbits at parturition (0hP, zero hour after birth when all the foetuses were born). The results are expressed as mean of 6 to 12 animals. Vertical lines show s.e. mean. Significance of differences between groups: C *vs* 28 days post coitum, $P<0.001$ (ct min^{-1} g^{-1} and ct/min per adrenal) and 30 days post coitum *vs* Ohp $P<0.002$ (ct min^{-1} g^{-1}).

similar pattern to that of PNMT. At 28 days post coitum, activity was lower than control values but higher than at 30 days post coitum when the results were expressed as ct/min per adrenal gland. Adrenaline content of adrenal gland declined significantly during the last few days of pregnancy but at parturition there was a tendency to return towards normal values (Table 1).

Discussion

From the present experiments, it is evident that the rate of adrenaline formation from noradrenaline declines throughout the duration of pregnancy, the fall being most marked at time of parturition. The decline in adrenaline content of the adrenal gland during late pregnancy is in keeping with the finding that PNMT activity is directly affected by the state of gestation.

If, during pregnancy, synthesis and catabolism are both functioning at a lower rate, there should be no significant change in monoamine content of adrenal glands. However, the observed decline in adrenaline concentrations in the adrenal gland suggests an accelerated release of this amine into the blood. This suggestion is strengthened by the studies of Zuspan & Zuspan (1973) who reported an increased output of catecholamines during late pregnancy and parturition, a finding confirmed by others (Parvez *et al.*, 1973a). A later study (Parvez *et al.*, 1973b) showed that during the last part of pregnancy (30 days post coitum) and parturition in rabbits, there was a 3–4 fold increase in plasma adrenaline and noradrenaline content. Furthermore, a few hours before parturition noradrenaline dropped to its minimum level whereas adrenaline remained higher, which points to some modification of PNMT activity at parturition.

The biochemical mechanisms responsible for the decline in PNMT activity appear to be connected with modifications in endocrine gland secretion. It has been reported that corticoids induce the synthesis of PNMT (Wurtman & Axelrod, 1966; Margolis, Roffi & Jost, 1966). Subsequent study of the response of extra-adrenal chromaffin tissue to corticosterone in organ culture (Coupland & MacDougall, 1966; Coupland, 1968; Barber, Coupland & Crowe, 1971) demonstrated that corticoids control the formation of adrenaline in cells which would otherwise store noradrenaline and that this effect is accompanied by induction of PNMT. Recently Mulay, Giannopoulos & Solomon (1973) have shown that the concentration of corticosterone in rabbit adrenal gland during pregnancy falls to one third of the value found in non-pregnant rabbits. The fall occurs 24 days post coitum and persists until parturition. These results may explain the low PNMT activity observed in our studies for at this time, lower concentrations of corticosterone are present to stimulate PNMT activity. A similar explanation may conceivably be applied to the lowered COMT activity which is also a methyl-transferase enzyme. An analysis of *in vivo* and *in vitro* effects of sex steroid hormones on monoamine catabolism has been made recently by Youdim, Holzbauer & Woods (1974) and others (Parvez *et al.*, 1975) in non-pregnant and pregnant rats. Oestrogens and progesterone alter COMT activity (Wurtman & Axelrod, 1966; Parvez *et al.*, 1975). Blood concentrations of oestrogens and progesterone change considerably during late pregnancy (Shaikh, 1971; Weist, 1970) and both hormones have been shown to exert an important influence on monoamine catabolism (Holzbauer & Youdim, 1973; Southgate, 1972) with progesterone acting as a stimulator and oestrogens as inhibitors.

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