Br. J. Pharmac. (1975), 53, 241-246

VARIATION IN ACTIVITY OF MONOAMINE METABOLIZING ENZYMES IN RAT LIVER DURING PREGNANCY

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1 Catechol-*O*-methyltransferase (COMT) and monoamine oxidase (MAO) activities in rat liver were measured during pregnancy, parturition and postpartum. Compared with activity in non-pregnant controls, both enzymes showed a significant decrease in activity which was most pronounced at day 18.

2 The metabolism of intravenously infused [^{3}H]-adrenaline to [^{3}H]-metanephrine and to [^{3}H]-acidic metabolites was also significantly depressed during pregnancy but had returned to control values by the 21st day.

3 The effects of reserpine and/or nialamide on hepatic COMT and MAO were studied in control and 20-day-pregnant rats. Their action on COMT activity differed in the two groups. MAO was inhibited to a similar extent in these groups whether the drugs were given separately or in combination.

4 It seems possible that the changes in endocrine function which occur during pregnancy are responsible for the observed alterations in enzyme activity.

Introduction

The effects of certain sex hormones on catecholamine release and metabolism in adult animals of a number of different species are well established (Pohorecky & Wurtman, 1971; Parvez & Parvez, 1972a; Holzbauer & Youdim, 1973). Variations in release into the blood stream, in urinary excretion and in adrenal content of catecholamines have recently been observed during pregnancy (Parvez, Gripois & Parvez, 1973; Parvez, Parvez & Gripois, 1973a) when profound hormonal changes are known to occur. Earlier, Cohen, Bitensky & Chayen (1965), using histochemical methods, noted variations in monoamine oxidase (MAO) staining patterns in the human endometrium during the menstrual cycle: enzyme activity appeared weak and confined to intercellular particles early in the proliferative phase, becoming progressively more intense and diffuse later in the cycle. Shortly before the onset of menstruation the particulate appearance was superseded by a diffuse staining of the whole cell. Quantitative evidence of these induced changes in MAO activity was later obtained by biochemical assay (Southgate, Grant, Pollard, Pryse-Davis & Sandler, 1968). Approximately ten-fold increases were observed on day 21 compared with earlier in the cycle. Variations in the MAO activity during the oestrus cycle have been noted in certain rodents' organs, including brain (Zolovick, Pearse, Boehlke & Eleftheriou, 1966; Cavanaugh & Zeller, 1967; Kamberi & Kobayashi, 1970; Holzbauer & Youdim, 1972).

The present experiments were designed to study the activity of the enzymes MAO and catechol-*O*-methyltransferase (COMT) in rat liver at different periods during the second half of pregnancy, at parturition and postpartum. In addition, three groups of rats were injected with reserpine and nialamide on the 20th day of pregnancy and the MAO and COMT activities in their livers were compared with those of non-pregnant females. The accumulation of injected [^{3}H]-adrenaline and its metabolism in pregnant rats were also studied.

Methods

Female rats (Sherman strain, 12-15 weeks old, 300 ± 50 g) were used. They were housed at a constant temperature (23° C) with natural day and night cycle and fed with commercial laboratory

food *ad libitum*. For fertilization, one male was kept with seven females from 19 h 00 min to 09 h 00 minutes. Pregnancy was verified by palpation 14 days later. Parturition occurred between day 21 and 22. For MAO and COMT analyses, animals were killed by neck fracture at 14 h 00 min on the specified day of pregnancy. Their livers were excised and kept in 0.9% w/v NaCl solution (saline) at -30° C.

[^{14}C]-Methyl-S-adenosyl methionine (sp. act. 50 mCi/mM) and [^{3}H]-(±)-adrenaline (sp. act. 4 Ci/mM were supplied by CEA, Gif sur Yvette, France. Tryptamine-[^{14}C]-bisuccinate (sp. act. 8.9 mCi/mM) was purchased from New England Nuclear Corporation, Boston, U.S.A. Normetanephrine (Calbiochem) and (±)-adrenaline (Rhone-Poulenc) were brought from Prolabo, Paris, France. Reserpine (Serpasil, Ciba) ampoules of 1 ml containing 2.5 mg suitable for subcutaneous administration, was supplied by Ciba Laboratories, Basel. Nialamide (in form of chlorohydrate, Pfizer Clinical Laboratories, Paris, France) was dissolved in sterilized olive oil. Control, as well as pregnant rats, were injected subcutaneously with reserpine (2.5 mg/kg) or nialamide (200 mg/kg). The doses for the combined administration were reserpine (1 mg/kg) + nialamide (100 mg/kg). In the pregnant rats, drugs were injected at 14 h 00 min on day 20 of pregnancy. This time was chosen to avoid stress during the last day of pregnancy or parturition.

Preparation of tissue for enzyme assay

Livers were homogenized in 40 volumes of ice-cold 0.9% KCl solution with glass homogenizers. Each homogenate was divided into two parts, one being stored for a short time at 1° C prior to MAO assay. The other was centrifuged for 30 min (50,000 *g*, 1° C) and the supernatant taken for COMT assay. Portions of homogenate and supernatant were boiled for 5 min and used as blanks.

Assay of MAO

The substrate used was tryptamine-[^{14}C]-bisuccinate (sp. act. 8.9 mCi/mM; Wurtman & Axelrod, 1963). The incubation mixture consisted of 0.44 nmole radioactive substrate in a volume of 50 µl, of 0.2 M phosphate buffer, pH 7.4, (200 µl) and tissue homogenate (100 µl). Otherwise, the procedure was as described (Wurtman & Axelrod, 1963) except that the final extraction was made into 3.5 ml toluene instead of six millilitres.

Assay of COMT

COMT was assayed using S-adenosyl methionine-[^{14}C] as methyl donor to one of the phenolic hydroxyl groups of adrenaline (Axelrod, 1962). The reaction mixture was prepared in glass tubes and consisted of 0.2 M phosphate buffer pH 7.9 (200 µl) 1% Mg Cl_2 (10 µl) 0.5 nmole adrenaline (50 µl), 0.44 nmole S-adenosyl methionine-[^{14}C] and enzyme preparation (100 µl). The tubes were closed and incubated in a water bath (37° C) for one hour. They were then cooled and 0.5 M borate buffer, pH 10 (0.5 ml), was added to stop the reaction. Toluene: isoamyl alcohol 3 : 1 (v/v) (3.5 ml) was added to each tube. After shaking for 25 min, tubes were centrifuged and 3 ml organic phase transferred to a scintillation vial containing 10 ml 'triton-toluene mixture' for counting.

Determination of [^{3}H]-adrenaline and its metabolites

The contents of one ampoule containing 1 mCi [^{3}H]-adrenaline were diluted to 3.33 ml with double-distilled water containing 0.001 M HCl. On the specified day of pregnancy, 1 ml of 0.9% saline containing 60 µCi of amine was infused into a saphenous vein over a period of 1 min under light ether anaesthesia. Twenty min later, the rats were killed by neck fracture and the livers excised immediately and homogenized in 8 ml of an ice-cold mixture of 4% trichloroacetic acid + 1% EDTA. [^{3}H]-Adrenaline was isolated on acid activated aluminium oxide (Euler & Lishajko, 1961) and eluted with 0.25 N acetic acid (2 ml). After centrifugation, 0.5 ml eluate was added to 10 ml liquid scintillator and counted for 10 minutes. [^{3}H]-Metanephrine and [^{3}H]-acidic metabolites were isolated according to the techniques of Axelrod & Tomchick (1960) and Axelrod, Weil-Malherbe & Tomchick (1959).

Results

COMT & MAO activity during pregnancy

Figure 1 shows COMT activity in rat liver at several periods of pregnancy, at parturition and postpartum. At day 18, activity was 44% lower than in non-pregnant controls. On day 20 of pregnancy, it was 27% higher than on day 18. It fell again on day 21 and at time of birth and was still low a few hours postpartum.

Figure 2 shows hepatic MAO activity at different stages of pregnancy. Compared to non-pregnant controls, activity was decreased by 24% on the 15th and 50% on the 18th day of pregnancy. At the 20th and 21st day, activity again increased, whilst a slight decline in activity

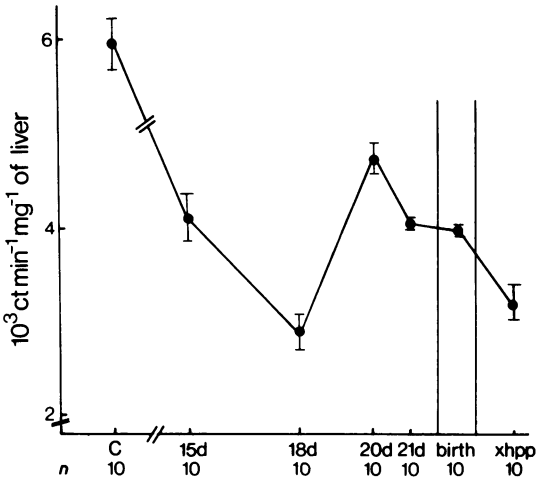

Figure 1 Catechol-*O*-methyltransferase (COMT) activity in rat liver during pregnancy, parturition and postpartum. *n* = number of animals used for each determination; C = non-pregnant females, 15d, 18d, 20d, 21d = days of pregnancy, and xhpp = 2 h after birth.

15d, 18d, 20d, 21d, birth and xhpp differ significantly from C($P < 0.001$) and 18d and 20d ($P < 0.001$).

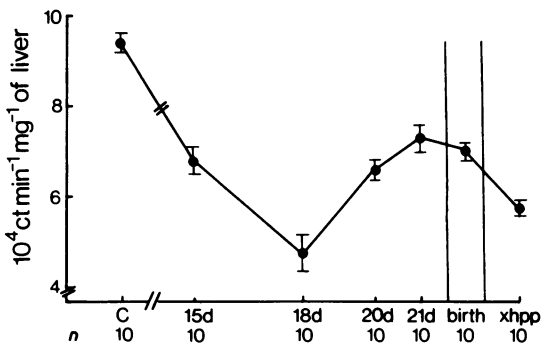

Figure 2 Monoamine oxidase (MAO) activity in rat liver during pregnancy, parturition and postpartum. *n* = number of animals used for each determination; C = non-pregnant females; 15d, 18d, 20d and 21d = days of pregnancy and xhpp = 2 h after birth.

15d, 18d, 20d, birth and xhpp differ significantly from C($P < 0.001$).

was observed during parturition which persisted for several hours afterwards.

The degree of degradation of intravenously infused [^{3}H]-adrenaline to [^{3}H]-metanephrine and [^{3}H]-acidic metabolites in male rats and in pregnant and non-pregnant females is compared in Figure 3. The amount of [^{3}H]-metanephrine and [^{3}H]-acidic metabolites found in the liver 20 min after an intravenous infusion of adrenaline was similar in males and in non-pregnant females. It was significantly lower in 18-day pregnant rats, but in 21-day pregnant rats was similar to control values.

Figure 4 provides a comparison between the effect of separate or combined administration of reserpine and nialamide on hepatic COMT activity in controls and in 20 days pregnant rats. In controls, reserpine inhibited COMT activity by 47%. In combination with nialamide, the same dose of reserpine did not produce any significant changes in COMT activity in pregnant rats. Nialamide decreased COMT activity in control and in pregnant rats by 23% and 40% respectively. The combined administration of reserpine and nialamide to non-pregnant females decreased COMT activity by 40%. However, similar treatment of 20-day pregnant rats produced only a 24% inhibition of COMT.

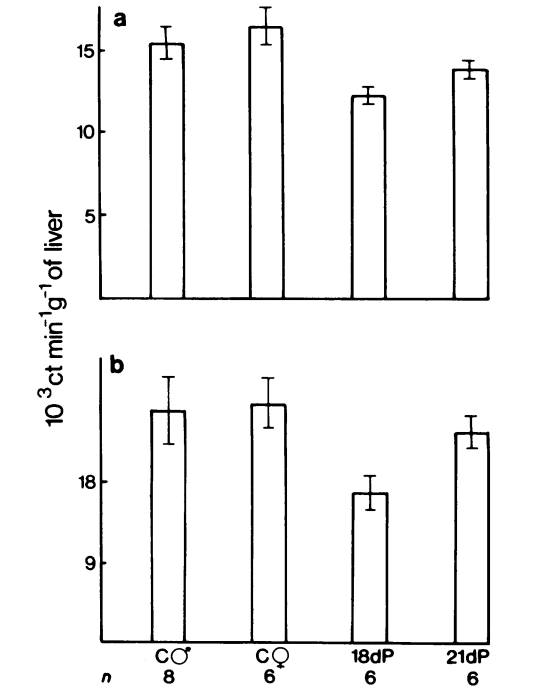

Figure 3 The transformation of [^{3}H]-adrenaline to (a) [^{3}H]-metadrenaline and (b) [^{3}H]-acidic metabolites in control male, female, 18 day pregnant (18dP) and 21 day pregnant rats (21dP). The animals received 60 μCi of tracer intravenously and livers were excised 20 min later.

Significant differences between female controls and 18dP were observed for metanephrine ($P < 0.01$) and acidic metabolites ($P < 0.01$).

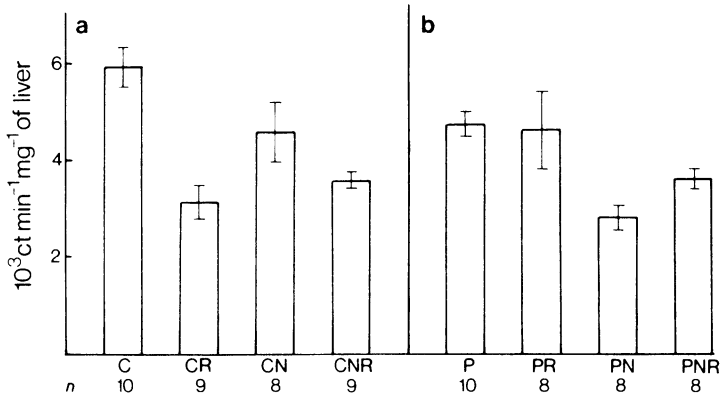

Figure 4 The effect of single or combined administration of reserpine (R) (2.5 mg/kg) and nialamide (N) (200 mg/kg) in (a) non-pregnant control (C) or (b) pregnant rats (P, 20 days) on catechol-*O*-methyl transferase (COMT) activity. *n* = number of rats.

Significance of differences between points:
C vs CR: $P < 0.001$; C vs CNR: $P < 0.001$; P vs PR: not significant; P vs PNR: $P < 0.01$; P vs PN: $P < 0.01$.

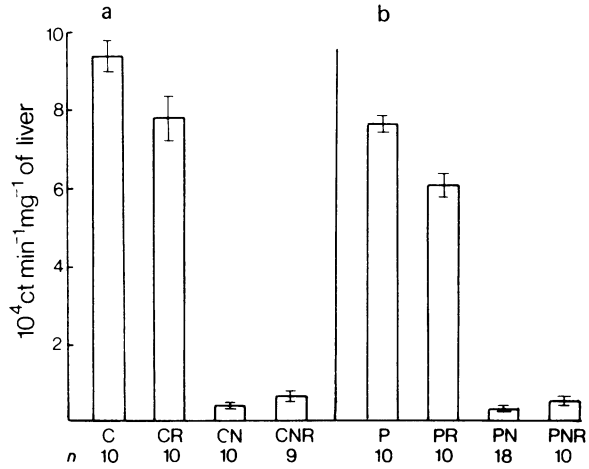

Figure 5 Monoamine oxidase (MAO) activity in (a) control (C) and (b) 20 day pregnant (P) rats after administration of reserpine (R) (2.5 mg/kg) or nialamide (N) (200 mg/kg). *n* =,number of rats.

Significance of differences between points:
C vs CR = not significant; P vs PR = not significant.

Figure 5 shows the effects of separate or combined administration of reserpine and nialamide on hepatic MAO activity in control and pregnant females. A single injection of reserpine (2.5 mg/kg) inhibited MAO activity by 17% in controls and 21% in pregnant rats. The administration of nialamide gave rise to inhibition of similar degree in both groups. Combined treatment resulted in 93% inhibition of MAO activity in both non-pregnant and pregnant rats, compared with untreated controls.

Discussion

The present results on pregnant rats provide presumptive evidence for hormones having an effect on catecholamine metabolizing enzymes. The decrease in activity of the two enzymes measured may conceivably be due to variation in the secretion of different hormones which are known to affect catecholamine metabolism. Evidence is available that corticosteroids and oestrogens inhibit catecholamine catabolism

(Callingham & Della Corte, 1972; Parvez & Parvez, 1972b, 1973; Holzbauer & Youdim, 1973). The naturally occurring changes in progesterone concentrations during the oestrous cycle are sufficiently large to influence MAO activity in various tissues including brain (Kamberi & Kobayashi, 1970; Holzbauer & Youdim, 1972; Youdim, Holzbauer & Woods, 1974). Some measure of the connection between MAO activity and progesterone production was provided by Holzbauer & Youdim (1973) who measured the progesterone content of ovaries and adrenal glands which synthesize and secrete about equal amounts of progesterone in the rat (Fajer, Holzbauer & Newport, 1971; Holzbauer & Godden, 1974). The extensive experiments of Ball, Knuppen, Haupt & Breuer (1972) who investigated the physicochemical interactions between oestrogens and catecholamines and their effect on methylation of catechol oestrogens, catecholamines and other catechols by COMT, point to an important role for oestrogens in the control of amine metabolism.

Previous studies on the urinary excretion of vanilmandelic acid during pregnancy and parturition (Parvez, Gripois & Parvez, 1973) are compatible with our present findings on MAO and COMT activities. Fluctuations in vanilmandelic acid excretion run parallel to MAO and COMT activities on the 18th, 20th and 21st day of pregnancy. Maternal levels of corticosteroid and oestrogens determined throughout pregnancy (Milkovic, Milkovic & Paunovic, 1973; Challis, Heap & Illingworth, 1973) also tend to agree with the present results. Urinary output, and release into the blood steam of both adrenaline and noradrenaline is persistently increased during the last days of pregnancy (Parvez *et al.*, 1973a and b). When MAO and COMT activities are low, they may well mirror a decrease in catecholamine catabolism during pregnancy. Preliminary results on the maintenance of glycogen stores in late pregnancy in different organs of the rat contrast with the low hepatic glycogen of early pregnancy which may be related to the present findings (Parvez, Parvez & Youdim, unpublished observations).

The effect of reserpine and nialamide on hepatic MAO and COMT are consistent with the findings of others (Euler, Bygdemann & Pearsson, 1971; Spector, Tarver & Berkowitz, 1972); however, there were differences between control and pregnant rats which appear to be the consequence of hormonal state and suggest that varying concentrations of different hormones in pregnancy counter the effects of reserpine and nialamide. Fluctuating concentrations of progesterone, oestrogens and glucocorticoids during pregnancy are well documented. Our results suggest that these changes may have functional importance in controlling the production of biogenic amines by regulating the activities of their catabolizing enzymes.

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(Revised July 26, 1974)