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DEVELOPMENTAL CHANGES IN THE ACTIVITY OF THE ENZYME CATECHOL-O-METHYLTRANSFERASE AFTER HYPOPHYSECTOMY OF FETAL RABBITS

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SUMMARY

The influence of fetal hypophysectomy by decapitation upon the development of the enzyme catechol-O-methyltransferase (COMT) in rabbit fetuses was studied. Fetal decapitation at day 27 of fetal life resulted in highly marked and significant increases of COMT activity in the kidney, liver and heart of the decapitated fetuses when compared with the values of non-operated controls. Decapitation at days 20 and 25 of fetal life also produced increases in the activity of COMT but a similar operation at day 23 resulted in decreased activity of this enzyme of 3-O-methylation of monoamines in liver and kidney. The results suggest that the normal function of the hypophyseo-adrenocortical system in the fetal rabbits serves as a rate limiting factor for the activity of the enzyme COMT. The inactivation of fetal adrenal cortex by decapitation results in increased COMT activity. These findings provide an important link between processes of monoamine metabolism and hypophyseo-adrenocortical hormones.

INTRODUCTION

The role of adrenocortical hormones in the regulation of monoamine storage, synthesis and metabolism has been studied extensively during the past decade [1, 2, 3]. Now it is well accepted that the constant supply of glucocorticoids in the adrenal portal circulation is essential for the induction of enzyme of adrenaline synthesis, phenylethanol-amine-*N*-methyltransferase in the adrenal gland of adult and fetal animals [4, 5]. Hypophysectomy of the adult rats results in marked inactivation of glucocorticoid production and this inactivation has a direct effect on the storage and synthesis of adrenaline [2, 3]. Our recent studies using inhibitors of glucocorticoid synthesis as well as adrenalectomy show that the activity of enzyme monoamine oxidase is highly increased when the glucocorticoids are significantly reduced [6, 7].

The present experiments were designed to see the influence of fetal adrenocortical function on the development of enzyme COMT in three peripheral organs. The activity of enzyme COMT in hypophysectomized and normal fetuses was compared at day 31 of fetal life, that is to say only one day before birth.

MATERIALS AND METHODS

White New Zealand strain rabbits were obtained from CNRZ, France. The animals were maintained at 21°C with exposures to natural light and darkness. The females were mated by keeping one male with one female under constant observation. After the copulation the females were separated in the individual cages and verified for pregnancy by palpation 14 days later. On days 20, 23, 25 and 27 post coitum, the females were anaesthesized by nembutal, and laporatomy was performed. The fetuses were hypophysectomized *in utero* by the techniques described previously [8, 9]. Some of the hypophysectomized fetuses were injected with adrenocorticotropic hormone, (ACTH-retard, Chaoy Laboratories, Paris) or hydrocortisone sodium succinate (Roussel Laboratories, Paris). The dose of ACTH was 0.5 IU and that of hydrocortisone was 0.5 mg per fetus. Both the hormones were administered intraperitoneally only once just after the decapitation. At the day 31 post coitum, the fetuses were excised by caesarean section. A maximum of 3 fetuses were hypophysectomized from one mother and the remaining fetuses were taken as sham or non-operated controls.

Assay of the enzyme COMT

On each specified day, the fetuses were dissected immediately and the heart, kidney and the liver were excised and transferred to a tube containing 0.9% ice cold KCl. The tissues were homogenized in ice cold 0.9% KCl (1 g/5 mg). The homogenates were centrifuged at 50,000 *g* for 30 min and the supernatant was taken as the enzyme preparation [10]. The method of Axelrod (II) utilizing S-adenosyl methione-^{14}C-methyl as methyl donor was used. The incubation mixture consisted of 0.2 ml of phosphate buffer (0.5 M at pH 7.9), 1-adrenaline (500 nmol in a volume of

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20 μl), 20 μl of Mg Cl_2 (1%), 0.2 ml of enzyme preparation and 50 μl of S-adenosyl methionine-^{14}C-methyl [10]. S-adenosyl methionine-[^{14}C]-methyl (S.A. 50 mCi/mmol) was obtained from C.E.A., Gif Sur Yvette, France. The incubation was carried out in a glass hemolyse tube at a temperature of 37°C for one h. After the uncubation the reaction was stopped by addition of 0.5 ml of borate buffer (1 M at pH 10) to each assay tube. The end products were extracted by addition of 4 ml of a mixture of toluene (3 volumes) and isoamyl alcohol (2 volumes). The tubes were shaken mechanically for 20 min. After centrifugation of the tubes at 5000 *g* for 5 min the organic phase was extracted into a vial containing 10 ml of Bray's phosphor scintillation solution. The extracted counts represented a direct linearity with the nmol of the end product transformed for one h of the incubation interval.

Statistical analysis

All the results were subjected to statistical analysis according to the method of Fisher (*t*-test). The mean values have been expressed with the standard errors (S.E.M.).

RESULTS

Figure 1 illustrates the influence of fetal hypophysectomy by decapitation on the day 20 of fetal life upon COMT activity of the kidney, liver and the heart determined at day 31 of fetal life. All these organs showed a marked and a significant increase in the enzyme activity after decapitation when compared to respective control values of non-operated fetuses from the same mothers. The highest increase in the activity was observed in the kidney but all the other increases were also statistically significant.

Figure 2 shows the activity of the enzyme COMT in the fetal rabbits hypophysectomized by decapitation on the day 23 of the fetal life. The enzyme activity in the kidney and the liver showed decreases but the heart did not show any important modification of statistical significance.

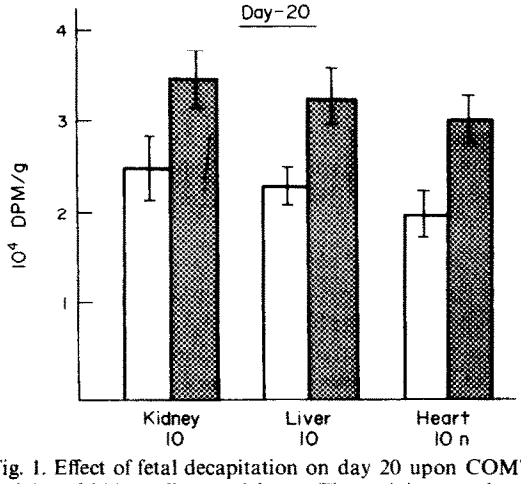

Fig. 1. Effect of fetal decapitation on day 20 upon COMT activity of kidney, liver and heart. The activity was determined on day 31 of fetal life. Statistically Control kidney vs Decapitated kidney, $P < 0.05$; Control liver and heart vs Decapitated liver and heart, $P < 0.02$ (tinted columns show decapitated values).

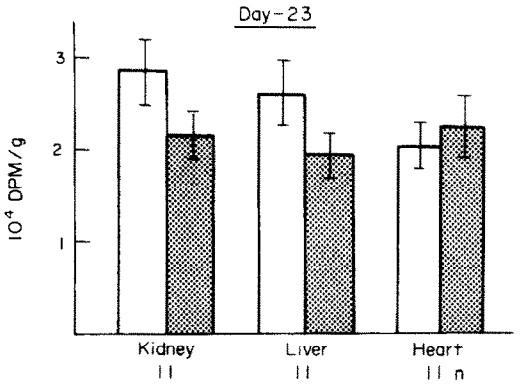

Fig. 2. Fetal decapitation on day 23 (tinted columns) and development of COMT activity at day 31. Statistically no group differed significantly.

The activity of the enzyme COMT in the kidney, liver and the heart of the rabbit fetuses hypophysectomized at day 25 of fetal age can be seen in Fig. 3. The activity of the enzyme COMT remained unchanged in the liver but the kidney and the heart showed increase in enzyme activity when compared to the values of non-operated controls of the same age. The increase in the activity of the COMT after decapitation at day 25 of fetal life was more marked in the heart than all the other organs studied.

Figure 4 shows the activity of the enzyme COMT in normal and hypophysectomized rabbit fetuses. The ablation of the head on day 27 of fetal life resulted in highly marked increases of the COMT activity of the kidney, heart and liver from respective non-operated values. Decapitation produced the maximum increase in the COMT activity of the kidney.

Figure 5 reports the effects of the treatments of ACTH and hydrocortisone of the fetuses decapitated at day 27 upon the development of the enzyme COMT in different peripheral organs. This short

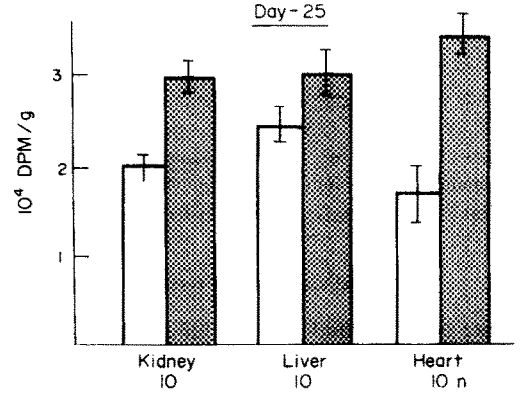

Fig. 3. COMT activity in normal (transparent columns) and decapitated fetuses at day 25 (tinted columns). Statistically normal kidney and heart vs decapitated kidney and heart, $P < 0.01$.

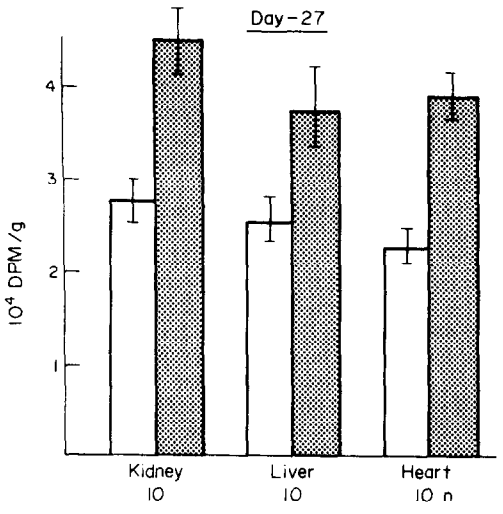

Fig. 4. Fetal decapitation at day 27 (tinted columns) and COMT activity. Statistically all organs differ significantly from operated values ($P < 0.02$).

treatment performed only once produced important decreases in the activity of the COMT which was highly increased after fetal decapitation. The dose of 0.5 IU of ACTH was more effective than 0.5 mg of hydrocortisone to induce declines in COMT activity as observed after the decapitation on day 27 of fetal age.

DISCUSSION

The results of the present investigation provide evidence that the normal development of the enzyme COMT is dependent on the presence of the pituitary gland. The increase in the activity of the COMT resulting after decapitation at days 20, 25 and 27 of the fetal life suggests that the hypophyseo-adrenocortical system serves as a rate limiting factor for the activity of the enzyme COMT which is the major enzyme responsible for 3-O-methylation of circulating monoamines. The specific role of hypophyseo-adrenocortical hormones to inhibit the activity of COMT can be confirmed by our results presented in Fig. 5 which show that even a very short treatment of ACTH or hydrocortisone to decapitated fetuses is effective to modify the COMT activity towards the normal values. The decrease which we observe after decapitation at day 23 can be explained on the basis of the developmental pattern of the fetal adrenal cortex. Recent studies show that day 23 of fetal life in rabbits is a very critical interval for the maturation and function of the adrenal cortex [12, 13]. Evidence is now accumulating that the placental transfer of glucocorticoids during the last interval of pregnancy is markedly modified and can be taken as one of the reasons for the decreased activity of the enzyme COMT on day 23.

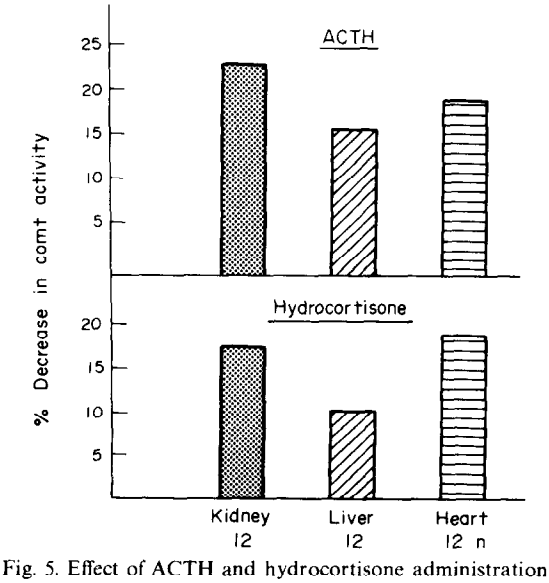

Fig. 5. Effect of ACTH and hydrocortisone administration to 27 days old decapitated fetuses. Both treatments decreased COMT activity in all the three organs studied. The decreases were more marked after ACTH treatment.

The results provided in this study do not permit to explain the biochemical mechanisms involved in provoking increases in the activity of the enzyme COMT. If one looks at the results of Wurtman and Axelrod[1] on the interrelations between glucocorticoids and the activity of the enzyme phenyl-ethanolamine-*N*-methyltransferase, it can be seen that the hormonal regulation of the process of *N*-methylation differs completely from that of 3-O-methylation. Glucocorticoids induce adrenaline synthesis by increasing the activity of the enzyme phenylethanolamine-*N*-methyltransferase in the adrenal medulla [14, 15]. The release of catecholamines into the circulation is also accelerated by perfusion of glucocorticoids [16]. However fetal hypophysectomy or adrenalectomy of the adult animals are accompanied by highly marked increases in the activity of the enzyme monoamine oxidase which is the other major enzyme of monoamine degradation [3, 6, 9, 17]. In the adult animals it is well established that the glucocorticoids stimulate adrenaline synthesis whereas they inhibit its metabolism [6, 9, 15]. This regulatory control of monoamine metabolism by glucocorticoid hormones also seems to be valid in the rabbit fetuses. This is a phenomenon of great interest for the body's reaction to stress since the normal levels of circulating corticoids stimulate adrenaline production and at the same time inhibit its metabolism so providing a huge supply of monoamines for better adjustment to external stimuli.

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