THE ROLE OF HYPOPHYSEO-ADRENOCORTICAL SYSTEM IN THE REGULATION OF ENZYME MONOAMINE OXIDASE IN RABBIT FOETUSES

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Received November 17,1972

Summary

The possible relationships of hypophyseo-adrenocortical axis in the evolution of enzyme monoamine oxidase (MAO) in rabbit foetuses from the age of 20 days was studied. The foetuses were deprived of their hypophysis by decapitation in utero at various ages. MAO was measured radiometrically in adrenals, kidneys, paraganglia, lung, liver and heart. There was a progressive rise in MAO activity determined on the 30th day in all cases in adrenals, kidneys and paraganglia following decapitation on the 20th day to 25th day. The activity in the above three organs remained highly significant from control levels even after decapitation on the 27th day. Lung, liver and heart demonstrated maximum activity after decapitation on the 23rd day. Administration of ACTH and hydrocortisone to the decapited foetuses for only once lowered MAO activity in adrenals, kidneys, heart and liver. The results provide evidence that the hormones of the hypophysis act as a rate limiting factor for MAO activity. Their deprivation upsets this rate limiting control resulting in marked rise in MAO activity.

Early observations suggest that hypophyseo-adrenocortical axis plays an essential role for the normal physiological growth of the adrenal cortex (1, 2, 3). Studies during recent years have brought out the fact that there is a reciprocal relationship between the foetal hypophysis and the foetal adrenal (4). The ablation of hypophysis in the adult (5) as well as in the foetus results in significant changes in several physiological and metabolic functions of the mammals. Hypophysectomy of the adult (6, 7) or foetal decapitaion (8) greatly affects adrenal stores of adrenaline and the activity of enzyme phenylethanolamine-N-methyl

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transferase (PNMT) declines markedly. Our recent observations (9, 10) provide evidence that the output of catecholamine metabolic bi-products is greatly enhanced following hypophysectomy. The inhibition of adrenal steroidogenesis results in significant increases in the activities of enzymes MAO and catechol-O-methyl transferase (COMT) (11, 12). Adrenalectomy at birth also produces increased MAO activity just in 10 days (13). The endocrine status of the rat foetus drastically changes by decapitation in utero (14). The evolution of many foetal enzymes could be modified by changes in hormone concentrations available to the mother or the foetus.

This study was performed to determine the differences in the development of enzyme of catecholamine degradation MAO in rabbit foetuses deprived of their hypophysis during variable ages. The decapited foetuses were also administered with ACTH and hydrocortisone and their MAO activity was compared with that of decapited foetuses to determine the importance of these specific hormones in regulation of MAO activity.

Materials and Methods

Tryptamine-^{14}C-bisuccinate (specific activity: 47.3 mC/mM) was purchased from New England Nuclear Corp., Boston, Mass., USA. The inorganic and organic solvents or compounds were bought from Merck, France. The adult rabbits of New Zealand strain were supplied by ETS. Ardenay, Sarthe, France.

The females were made pregnant in the laboratory by keeping the male with females for short time under constant watching. They were separated just after copulation and verified for pregnancy by palpation on the 14th day. The females were operated under Nembutal anaesthesia and laparotomy was performed to see the physical condition of the foetuses. Only the rabbit with light pink colour of the foetuses was used for foetal decapitation. Foetal decapitation was performed according to the methods described in the past (15, 16). A small suture was performed in the uterus at the tip of the foetal head. The head was pushed out carefully and cut by cat gut. The uterus as well as the foetus were well stitched. The maximum number of foetuses decapited were 4 from each mother. 1.2 UI of ACTH or hydrocortisone in 0.9 % saline was injected to the separate groups of de-

capited foetuses just after the operation. The foetuses were taken out at 0 hour at the end of 30th day by cesarean under Nembutal anaesthesia. Two types of controls were taken: (C) from the mother not operated for decapitation, (CD) the brothers of the decapited foetuses from the mother operated for decapitation. All the foetuses aged 30 days at the time the tissues were excised. The tissues were immediately taken out from the foetus and kept in ice cold 0.9 % KCl for short time before enzyme assays.

MAO was assayed by a slight modification of the technique of Wurtman and Axelrod (17). Tryptamine-C^{14}-bisuccinate instead of tryptamine-C^{14}-hydrochloride was utilized. This modification did not change the precision of the assay up to the slightest extent. The tissues were homogenized in chilled 0.9 % KCl in a concentration of 2 mg/ml. The incubation mixture consisted of 400 µg of homogenate in 0.2 ml, 0.1 mµ mole of tryptamine-C^{14}-bisuccinate in volume of 0.1 ml and 0.25 ml of phosphate buffer, 0.2 M at pH 7.4. The rest of the procedure was the same as in the original method. Duplicate samples were prepared for each determination. The enzyme activity is directly expressed in DPM/mg of tissue as toluene extraction media represented a proportionality in DPM extracted and the µ moles of C^{14}-indole acetic acid transformed during 20 minutes of incubation.

Statistical differences were compared by Fisher's Student "t" Test. The mean values are expressed with standard errors of the means.

Results

Fig. 1. shows the activity of MAO in DPM/mg of tissue in adrenals and kidneys of control and decapited foetuses. The activity of MAO was slightly lower in controls (C) taken out from unoperated mothers at the end of 30th day. The unoperated brothers of the decapited foetuses (CD) indicated little rise but without any significance. The foetuses following decapitation at 20th, 23rd and 25th day showed a progressive rise in MAO activity from the control values (CD). The activity in adrenals was 45 %, 110 %, 192 % and 110 % higher than their controls (CD) at 20th, 23rd, 25th and 27th day of decapitation respectively. In kidneys MAO activity rose by 21 % following decapitation on the 20th day, 52 % on 23rd day, 85 % on 25th day and 34 % on 27th day. The maximum effects of decapitation to increase MAO activity was found following decapitation on 25th day in both the organs.

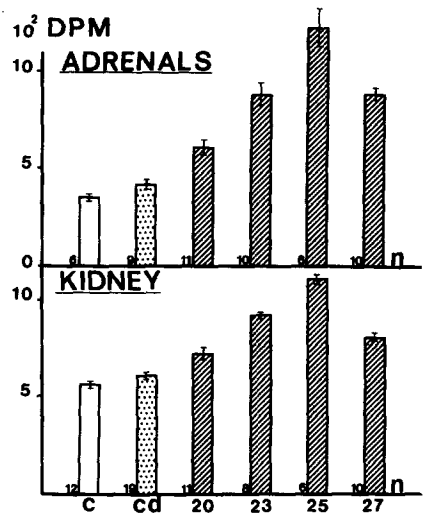

Fig. 1.

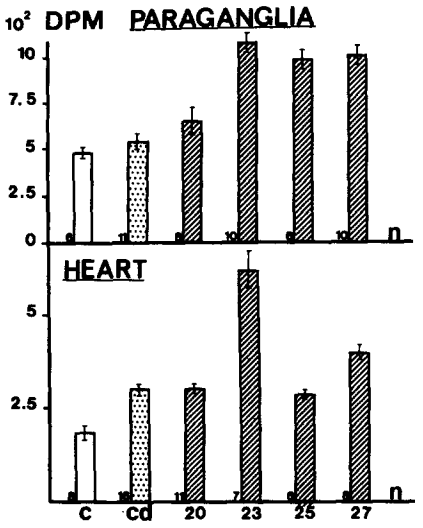

Fig. 2.

Fig. 1. The activity of enzyme MAO in adrenals and kidneys expressed in DPM/mg of tissue . C(Controls from unoperated mother), CD(Controls from the mother operated for decapitation), 20, 23, 25, 27 age of decapitation. The mean values are expressed with SE. n(Number of cases)

Fig. 2. MAO activity in paraganglia(extra adrenal chromaffin tissue) and heart in DPM/mg of tissue. C (Controls from unoperated mother), CD(Controls from operated mother), 20, 23, 25, 27 (Decapitation age). n(Number of determinations)

All these increases were highly significant from their respective control values ($P < 0.001$).

Fig. 2. indicates activity of MAO in paraganglia (extra adrenal chromaffin tissue) and heart in DPM/mg of organ weight. The enzyme activity showed a rise of 41 % and 117 % in the paraganglia following decapitation on 20th and 23rd day. At 25th and 27th day MAO in paraganglia was 98 and 102 % higher than controls (CD). (CD & 20 days $P < 0.02$, CD & 25 days $P < 0.001$). The cardiac MAO did not change on the 20th day of decapitation. The cardiac MAO was 107 % higher following decapitation on the 25th day ($P < 0.001$). The decapitation on the 27th day increased the activity significantly($P < 0.001$). The effects of foetal decapitation on the evolution of enzyme MAO in lung and liver are given in Fig. 3. The enzyme activity rose by 37 % in both the

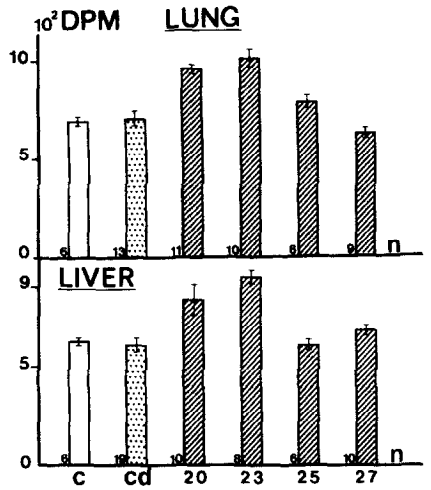

Fig. 3. The activity of MAO in lung and liver of C(controls from unoperated mother), CD(controls from operated mothet), 20, 23, 25, 25 days decapited rabbit foetuses. The means are given with SE. n(Number of cases)

organs after decapitation on the 20th day. The decapitation on the 23rd day elevated the activities by 42 % and 56 % in lung and heart respectively. The rises at 20 and 23rd day were highly significant ($P<0.001$). Afterwards the decapitation on the 25th day and 27th day did not change the activities to the extent of statistical significance in both the organs.

Table 1. shows the effects of ACTH and hydrocortisone administration to foetuses decapited at 23rd and 25th day. The administration of both the hormones only once declined MAO activity but the declines were variable to each specific tissue and the day of administration.

Discussion

The results show that inactivation of the adrenal cortex by deprivation of hypophyseal hormones results in marked increases in MAO activity. The maximum rise in activity was observed in the adrenals of the foetuses decapited at the age of 25 days. This provides a fair evidence that adrenal gland is the target organ affected by decapitation. The variations in age of decapitation to produce maximum rise in MAO activity in different tissues appears to be attributed to the hormonal specificities of specific MAO in different tissues. It is well accepted that MAO exists in mutiple forms

Table 1. Decline in activity of MAO following administration of ACTH or Hydrocortisone to decapited foetuses.

Organ	23 D + ACTH	25 D + ACTH	23 D + Hydrocortisone
Adrenals	10 % (5)	31 % (8)	No Change (8)
Kidneys	No Change (5)	20 % (8)	No Change (8)
Heart	47 % (5)	No Change (5)	47 % (8)
Lung	35 % (5)	No Change (5)	No Change (8)

23 D, 25 D (Age of Decapitation). ( ) Number of Determinations.
1 mg of Hydrocortisone(Ciba) or 1.2 IU ACTH was injected to the foetuses just after decapitation.

and protein specificity of each differs from organ to organ. The induction and regulation of each MAO differ from that of other (18). The hypophysea-adrenal homeostasis exists during foetal age and decapitation upsets this mechanism (4). The hormones of the adrenal cortex are well investigated and sufficient data is available (19, 20) that they regulate many enzymes of metabolic significance. Our previous results (11, 12) show that corticoids in normal animals inhibit MAO and COMT activities. The inhibition of these hormones by drugs or their ablation by adrenalectomy (12, 13) increases the activities of MAO and COMT. The present results are in agreement to previous findings as decapitation greatly lowers the adrenal corticoidogenesis. (4). The replacement therapy with ACTH or hydrocortisone declines the rise in activity to a good extent.

The biochemical interpretation for increased MAO activity due to lower level of corticoids seems opposite to the induction of PNMT (6, 8). It appears that corticoids limit MAO activity by interfering with protein synthesis. Hydrocortisone in very low concentrations inhibits MAO in vitro. (11). Recent studies provide evidence that the changes in enzyme activity are thought to occur on the amount of enzyme protein present in the tissues, and consequently the regulating factors. The regulating factors affect the rate of synthesis as well as break down of enzyme protein (21). In some cases the formation of apoenzyme occurs quite independently of the enzyme protein (22). The genetic formation for the synthesis of specific enzyme protein originates from the DNA of the structural gene, is transcribed to the messenger RNA, and then goes to the template RNA of the ribosomes where activated amino acids are synthesized to the specific protein molecule (23). The biosynthesis of active enzyme protein might eventually be dependent on a sufficient amount of hormonal stimulated effectors or other cofactors. It

is suggested that corticoids inhibit MAO by interfering the regulating factors for enzyme protein synthesis as described above.

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