Adrenomedullary Control of Release of Adrenaline and Noradrenaline in Plasma of Foetal and New Born Rabbits

The localization and function of catecholamines for maintaining a physiological homeostasis, especially in stressful situations, have been extensively studied[1,2]. In the adrenal glands of foetal and new-born animals, the ratio noradrenaline/adrenaline was found to be higher than in the adult[3-5]. The study of adrenal catecholamines of the developing rabbit foetus shows that after 24 days of gestation the adrenaline content far exceeds that of noradrenaline[6]. Certain patho-physiological conditions have been found to cause increased release of catecholamines from denervated adrenal medulla[7-10]. This non-nervous and direct stimulation of the adrenal medulla has been found more easily demonstrable in foetal and new-born animals than in adults[11]. Recent abservations in the foetus and new-born of different species have shown marked variations in catecholamine storage[12,13]. The enzymes of catecholamine biosynthesis and metabolism have also been reported to fluctuate during gestation[14-16]. The present study reports the levels of plasmatic adrenaline and noradrenaline in foetal and new-born rabbits. The activity of enzyme phenylethanolamine-N-methyl transferase (PNMT) in adrenals was also determined.

Materials and methods. Female rabbits of New Zealand strain were made pregnant under constant observation and separated in individual cages after fertilization. On the specified day of pregnancy, laparotomy was performed under nembutal anaesthesia and foetuses were taken out immediately. The new-born rabbits were taken immediately after birth. The foetal age in XhP groups was between 4–8 h post partum. 1 ml blood from each foetus or new-born was taken from the jugluar vein in a heparinized syringe. This procedure prevented hemolysis and shock. The estimation of catecholamines in several control experiments was performed to test the validity of this method. The pooled blood from several animals was mixed with 4 ml of 3% sodium thiosulfate and 2% sodium fluoride mixture[17]. The mixture was centrifuged at 15,000 *g* at 2°C for 10 min and plasma was poured gently in a small beaker. The plasmatic adrenaline and noradrenaline were adsorbed on aluminium oxide[18] using batch process[19,20]. The acetic acid elutes were estimated flurometrically[21,22].

Enzyme PNMT was assayed utilizing ^{14}C-S-adenosyl methionine as methyl donner[23,24]. The incubation mixture was prepared as described in the original methods[23,24]. The enzyme acitvity is expressed in dpm extracted after 1 h incubation All the results are expressed with standard errors of the mean values (SEM).

Results. The development in release of adrenaline and noradrenaline in plasma of foetal and new-born rabbits is illustrated in Figure 1. The mean values ± SEM are given in µg/l of plasma. The foetuses at the age of 25 days demonstrated the highest concentration of plasmatic adrenaline. Respective declines of 52 and 74% on 28th and 30th day of gestation were observed in plasma adrenaline as compared to 25 days. All these declines were statistically significant ($P < 0.001$). On the 31st day of pregnancy, the adrenaline level was 42% higher than at 30 days, but it was 62% lower than at 25 days ($P < 0.001$). At parturition, a maximum decline of 92% was observed. This decline, compared with 25 days, was highly significant ($P < 0.001$). A few hours after parturition, the plasmatic adrenaline started to increase. The noradrenaline in plasma on the 25th day of pregnancy had a mean of 2.6 µg/l. There was 36% increase in this value in the plasma of 28 days foetuses ($P < 0.001$). During 30th and 31st day respective declines of 50% and 129% compared to 28 days were observed. These declines were highly significant ($P < 0.001$). At parturition, the plasmatic noradrenaline showed a $2^1/_2$-fold increase from the value at 31 days ($P < 0.001$). A few hours after birth, lower levels of plasma noradrenaline were observed[25,26].

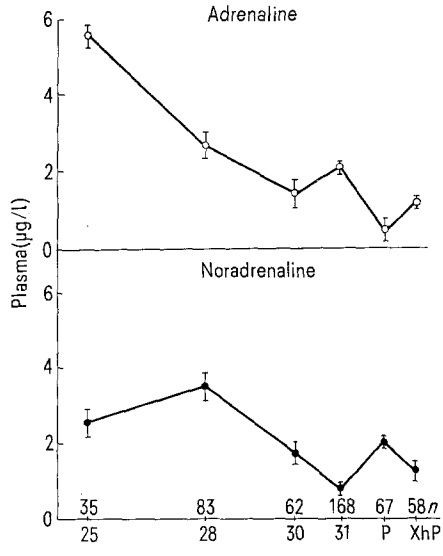

Fig. 1. The levels of plasma adrenaline and noradrenaline in foetal and new-born rabbits. 25, 28, 30, 31 (foetal age), P (parturition), XhP (4–8 h after birth). *n* (number of animals utilized for each group.)

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Figure 2 shows the activity of enzyme PNMT in the adrenals of foetal and new-born rabbits. The rate of methylation of noradrenaline to adrenaline was very high in the foetal adrenals of 25 days. This value declined by 37% in foetuses of 28 days ($P < 0.001$). At the age of 30 days, the foetal adrenals had the same level of PNMT activity as at 28 days. At 31 days an increase of 64% from that of 30 days occurred ($P < 0.001$). At the time of parturition, the methylation of noradrenaline to adrenaline was at the lowest level compared to all the values throughout the determination period ($P < 0.001$). At few hours post parturition, the enzyme activity showed nearly 3-fold increase from that at parturition.

Discussion. The active synthesis of adrenaline by foetal adrenals and paraganglia (extra adrenal chromaffin tissue) during last term of pregnancy in rabbits was shown by BRUNDIN et al.[27,28]. The results of the present investigation indicate marked variations in the release of adrenaline and noradrenaline in plasma of foetal and new-born rabbits. The determination of plasma noradrenaline and activity of enzyme PNMT in the adrenals shows that the release of both adrenaline and noradrenaline in the blood was directly affected by changes in noradrenaline methylation to adrenaline. Previous study has confirmed that most of the adrenaline in circulation is the product of adrenomedullary secretion[29]. The modifications in catecholamine release in blood and altered pattern of PNMT activity could be attributed to changes in concentration of corticosteroids[30–32] which are essential for induction of enzyme PNMT[33]. It was reported that hypophysectomy in animals caused a reduction in adrenal content of adrenaline and PNMT activity[34]. Similar effects were also confirmed in the foetus[14,15]. The reduction in both PNMT activity and adrenaline content of adrenals of hypophysectomized animals could be prevented after pretreatment with corticosteroids[34,35]. It was also shown that release of catecholamines in the circulation could also be greatly affected by changing the concentration of corticosteroids[36,37]. In the adrenal medulla, high concentrations of corticosteroid hormones are supplied via the direct vascular connections which have been found between the cortex and medulla[38]. Therefore any change in corticosteroid level will directly influence catecholamine biosynthesis and release. The detailed study about factors affecting release of catecholamines from adrenal medulla under different physiological conditions is extensively studied in the foetus by COMLINE et al.[10,12].

It is concluded that the changes in adrenaline and noradrenaline in plasma observed in foetus and new-born rabbits are induced directly by rate of adrenaline methylation from noradrenaline.

Zusammenfassung. Die Plasmaspiegel von Adrenalin und Noradrenalin bei Kaninchen-Foeten und neugeborenen Kaninchen wurde einige Tage vor Beendigung der Schwangerschaft sowie unmittelbar postpartal bestimmt. Die Resultate zeigten, dass die verschiedenen Plasma-Catecholaminwerte auf den Metabolismus der Catecholamin-Synthese zurückzuführen sind.

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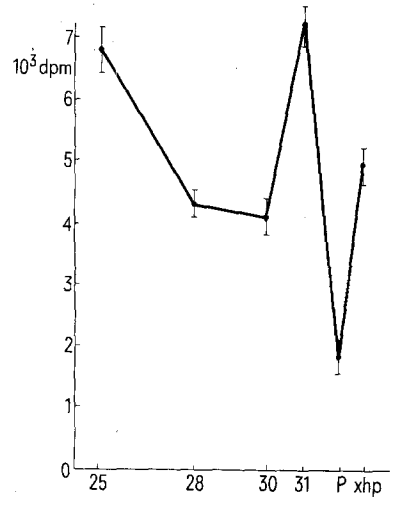

Fig. 2. Activity of enzyme phenylethanolamine-N-methyl transferase (PNMT) in adrenals of new-born and foetal rabbits. 25, 28, 30, 31 (days of pregnancy). P (parturition), XhP (4–8 h post parturition). All the groups contained at least 20 foetuses or new-born animals.

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Creatinine: A Precursor of Methylguanidine

We have previously demonstrated[1] that serum and urinary levels of methylguanidine (MG) are increased in uremia. Chronic administration of this substance produces an uremic-like syndrome in dogs[2] raising the possibility that MG is responsible for many of the manifestations of chronic renal insufficiency. The present investigation was designed to study the metabolic pathway for the synthesis of MG in the rat. In the present communication we have found that creatinine, for a long time believed to be metabolically inert[3], is a precursor of MG.

MG was determined by ion exchange column chromatography[1] and a modification of the VOGES-PROSKAUER reaction[4]. The biogenesis of MG was first studied by determining its urinary excretion in animals given unlabelled potential precursors. Sprague-Dawley rats, weighing between 150 and 300 g, were injected i.p. with