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Plasma catecholamine concentrations in lambs and calves during the perinatal period

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Summary. Concentrations of the plasma catecholamines (CA), adrenaline, noradrenaline and dopamine, were measured using a specific and sensitive radioenzymatic assay in 7 ewes and their single lambs chronically catheterized *in utero* between days 127 and 145 of gestation and during the first week of postnatal life. These concentrations were also measured during the first month after birth in 18 Holstein x Friesian calves, of which 9 were born spontaneously at term (278 days) and 9 by caesarean section on day 260 of gestation.

Plasma CA concentrations in ewes showed no significant variations during the sampling period. Plasma adrenaline, noradrenaline and dopamine concentrations in lambs at birth rose significantly to 1 800 $\pm$ 520, 3 782 $\pm$ 781 and 575 $\pm$ 90 pg/ml, respectively. These values remained higher than those measured in ewes until 2, 24 and 48 h after birth, respectively.

On days 5 and 10 after delivery, plasma dopamine and noradrenaline concentrations were higher in calves delivered by caesarian section than in those born spontaneously at term, while plasma adrenaline concentration was never different between the two groups.

These results indicate that the surge in plasma CA concentration normally occurring at birth might be delayed *post partum* in animals delivered before term. This might partly explain why these animals were less resistant to stress during the first days of postnatal life.

Introduction.

Catecholamines (CA) play a major role in a variety of neonatal adaptations, including non-shivering thermogenesis (Girardier and Seydoux, 1971 ; Eales and Small, 1980), glucose and lipid homeostasis (Bassett, 1970 ; Fowden, 1980), cardiovascular-renal changes (Geis *et al.*, 1975 ; Buckley *et al.*, 1979), lung maturation (Lawson *et al.*, 1978 ; Brown *et al.*, 1983) and regulation of sodium and water metabolisms (Güllner, 1983). CA release by foetal ovine (Comline and Silver, 1961 ; Jones and Robinson, 1975), foetal bovine (Comline and Silver, 1966) and foetal equine (Comline and Silver, 1971) adrenal glands has been studied *in vivo*. However, in these studies CA's were measured by a relatively insensitive bioassay using the blood pressure response of the anaesthetized rat (Outschoorn,

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1952) or a non-specific fluorimetric method (Von Euler and Floding, 1955). Little information exists in the literature in regard to plasma CA concentrations in chronically catheterized foetal or newborn animals (Palmer *et al.,* 1984). The development of a specific and sensitive radioenzymatic assay for CA (Da Prada and Zürcher, 1976) and of carefully standardized methods of foetal blood collection (Barlet, Davicco and Lefaivre, 1982) facilitates such studies during the perinatal period in lambs and calves.

In the present work, we have measured plasma CA concentrations in chronically catheterized foetal lambs *in utero,* in their dams before and after birth, and in calves born spontaneously at term or delivered by caesarian section before term.

Material and methods.

Animals.

Sheep : We used seven primiparous Limousine ewes weighing 57 $\pm$ 4 kg mated with a ram of the same breed ; the ewes were of known gestational age and carried a single foetus detected by radiography on day 110 of gestation. The ewes were housed in individual pens indoors at 14-16 °C and fed hay and grain concentrate ; they had free access to tap water and were accustomed to handling and blood sampling during the month before the experiment began. Between days 110 and 116 of gestation, a sterile heparinized catheter was chronically implanted into the carotid artery of each foetus under halothane anaesthesia (Barlet, Davicco and Lefaivre, 1982). At the same time, another catheter was chronically implanted into the right jugular vein of each ewe. The dead volume of each catheter was measured before surgery and the arterial and venous catheters were filled with sterile saline containing heparine (2 500 IU/ml) and thiomersal (0.02 ml/ml of a solution containing 1 g of thiomersal/l of 90 % ethanol) (Barlet, Davicco and Lefaivre, 1982). The first blood sample was taken 10 days after surgery. At the first symptoms of parturition, the foetal catheters were cut and sealed with flame to prevent any blood loss during delivery. They were re-opened after birth and used again for blood sampling in the lambs. Each lamb was born alive between days 144 and 146 of gestation. The birth weight of the 7 lambs (3.1 $\pm$ 0.3 kg) was not different from that of the unoperated single lambs of the same genotype from primiparous ewes of the same breed. After birth each lamb was left with its mother and suckled *ad libitum.*

Calves : 18 Holstein $\times$ Friesian calves were used. Nine were males born spontaneously at term (278 days of gestation). The other 9 animals (8 males and 1 female) were delivered by caesarian section on day 260 of gestation, immediately after maternal stunning with a concussion stunner before exsanguination. The calf was removed from the uterus within one minute after the mother was stunned ; it was placed under a warming blanket at 39 °C for 24 h and fed 4 times per day with 1 l of thawed bovine colostrum. Control calves born at term were fed maternal colostrum during the day following birth and thereafter a milk replacer twice per day. The birth weight of calves delivered before term

(34.1 ± 1.7 kg) was lower ($P < 0.05$) than that of control calves born at term (40.6 ± 0.9 kg). This difference still persisted at 30 days (45.6 ± 2.9 *vs* 64.4 ± 2.0 kg ; $P < 0.01$). Blood samples were obtained from calves through an heparinized catheter implanted into the right jugular vein at least 12 h before each sampling.

Catecholamine assay.

Blood samples (1 ml) were collected into plastic syringes containing heparin (10 IU/ml). Immediately after collection the samples were transferred to chilled polypropylene tubes and centrifuged for 2 min at 3 000 × g at 4 °C. The plasma was collected and stored at − 20 °C until assay performed during the month after the last blood collection in lambs or in calves. We used 200 µl of plasma to simultaneously assay adrenaline (A), noradrenaline (NA) and dopamine (DA) in duplicate by the radioenzymatic method of Da Prada and Zürcher (1976). Briefly, the CA were converted into their O-methylated labelled derivatives by catechol-O-methyl transferase in the presence of tritiated-S-adenosyl-methionine (New

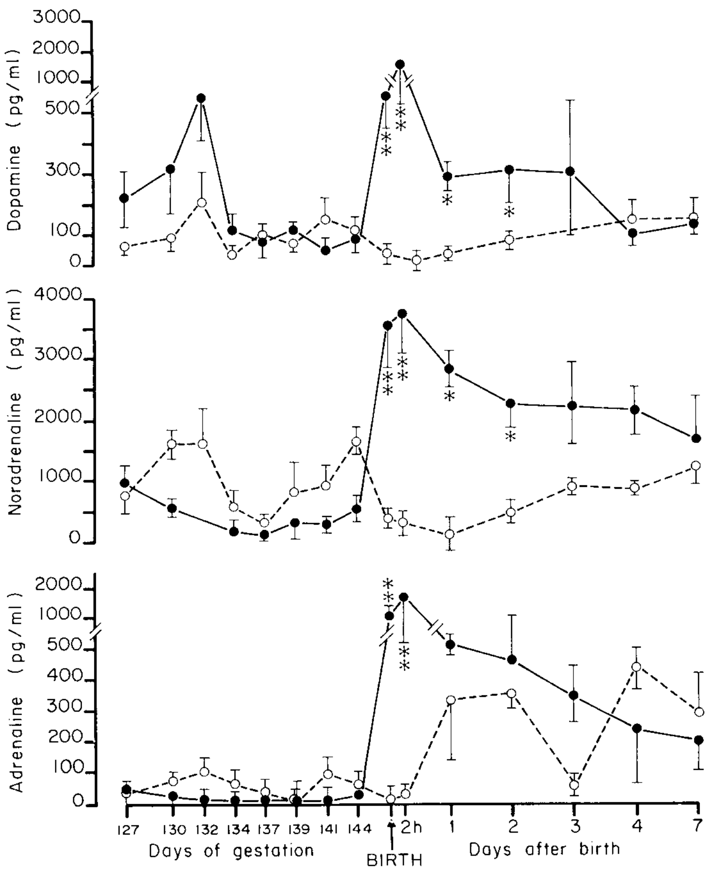

FIG. 1. — *Plasma catecholamine concentrations in 7 ewes* (dashed line) *and their 7 lambs* (black line) *during the last 19 days of gestation and the first postnatal week* (mean ± SEM ; $^*P < 0.05$, $^{**}P < 0.01$; comparison between lambs and ewes).

England Nuclear France, Paris). The radioactive methylated derivatives were then extracted and separated by thin-layer chromatography. ^3H-methoxytyramine was scraped off and assayed directly for its radioactivity. The tritiated A and NA were first converted to vanillin by periodate cleavage and then extracted into toluene (Bühler *et al.,* 1978) ; their radioactivity was measured by a Beckman LS 6 800 liquid scintillation counter.

Basal DA values were at least 3 times, and A and NA values 5-6 times, the blank values. Internal standards were always measured in each assay. Assay sensitivity was 1 pg for A, 1.5 pg for NA and 4 pg for DA. Inter and intra-assay variabilities were 6 and 2 % for A, 6 and 3 % for NA and 7 and 10 % for DA, respectively.

To assess foetal status, blood pH was measured using a PHM 73 blood gas monitor (Radiometer, Copenhagen) on each blood sample collected from the foetuses. The mean pH value was 7.35 $\pm$ 0.02 (n = 10) between days 125 and 145 of gestation and 7.29 $\pm$ 0.03 (n = 7) immediately after birth.

The results were expressed as the mean $\pm$ SEM. The statistical significance of the differences observed between groups was calculated using the Mann-Whitney U-test of variance analysis.

Results.

The DA, NA and A concentrations in ewe plasma showed no significant variation during the experimental period (fig. 1).

Before parturition, plasma DA concentrations were never different in ewes and their foetuses (table 1 ; fig. 1). They increased sharply in lambs at parturition time (575 $\pm$ 90 pg/ml) and remained significantly higher than in ewes until 48 h post partum (fig. 1).

TABLE 1

Plasma catecholamine concentrations (pg/ml) *in 7 ewes and their 7 lambs between days 125 and 145 of gestation and during the first week of postnatal life.*

		Days	
		125-145	1-7
DA	Ewes	102 $\pm$ 17	84 $\pm$ 19
	Lambs	194 $\pm$ 64	415 $\pm$ 162*
NA	Ewes	1 020 $\pm$ 183	1 315 $\pm$ 188
	Lambs	621 $\pm$ 156*	2 774 $\pm$ 414**
A	Ewes	91 $\pm$ 28	269 $\pm$ 89
	Lambs	19 $\pm$ 5*	697 $\pm$ 213*

Results are presented as means $\pm$ SEM of values indicated in figure 1.

DA : dopamine ; NA : noradrenaline ; A : adrenaline ; * $P < 0.05$; ** $P < 0.01$; comparison with maternal values.

Before parturition, mean plasma NA and A concentrations were lower in foetuses than in ewes (table 1). Foetal plasma NA and A concentrations increased sharply at birth up to 3 782 ± 781 pg/ml and 1 800 ± 520 pg/ml, respectively. These increases occurred during labour since plasma NA and A concentrations, measured just before labour in 4 foetuses, were 704 ± 53 pg/ml and 27 ± 5 pg/ml, respectively. A and NA concentrations remained higher in foetal plasma than in maternal plasma until 2 h and 48 h post partum, respectively (fig. 1).

In calves born spontaneously at term, plasma DA concentrations (mean value : 380 ± 57 pg/ml ; n = 36) showed no significant variations between days 1 and 30 post partum. On day 1, they were not significantly different from those measured in calves delivered by caesarian section before term. However,in the latter, these concentrations increased from 530 ± 100 pg/ml to 2 800 ± 520 pg/ml ($P < 0.01$) between days 1 and 5 post partum and until day 10 remained higher than in calves born spontaneously at term (fig. 2).

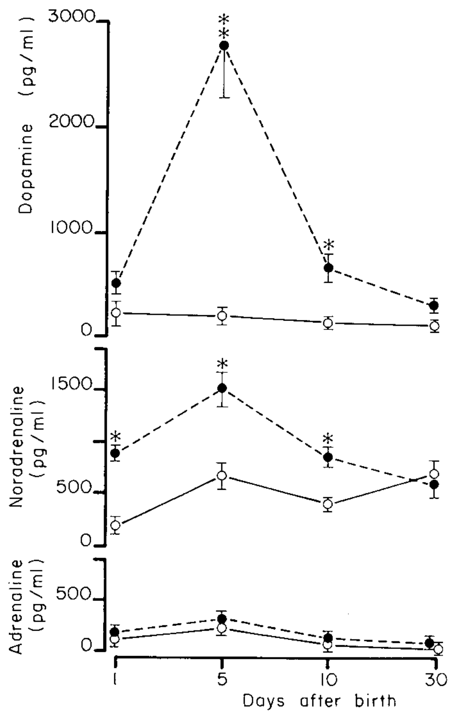

FIG. 2. — *Plasma catecholamine concentrations in 9 calves born spontaneously at term* (278 days ; black line) *and in 9 calves delivered by caesarian section on day 260 of gestation* (dashed line). (Mean ± SEM ; *$P < 0.05$, **$P < 0.01$; comparison between the two groups of calves).

In both groups of calves, plasma NA concentrations increased ($P < 0.05$) between days 1 and 5 post partum. Twenty-four hours after birth, plasma NA concentrations were higher in calves delivered by caesarian section before term (900 ± 80 pg/ml) than in those born spontaneously at term (200 ± 60 pg/ml). This difference persisted until day 10 post partum (fig. 2).

Plasma A concentrations showed no significant variations in either group of animals (fig. 2). Mean plasma A concentration measured in calves born spontaneously at term (114 ± 19 pg/ml ; n = 36) was not different from that measured in those delivered by caesarian section before term (121 ± 30 pg/ml ; n = 36).

Discussion.

To our knowledge, this is the first time that plasma CA concentrations have been measured in the maternal and foetal plasma of the ovine species both before and after birth. Philippo, Lawrence and Mellor (1975) measured plasma A and NA concentrations in 6 ewes and their lambs and in pregnant cows from 7 days before until 0.5 day after birth. They found that NA concentrations in ovine and bovine maternal plasma and in ovine foetal plasma reached a peak just before birth or at the time of birth. Jones and Robinson (1975) reported an undetectable baseline plasma CA concentration in a similar ovine preparation using a trihydroxyindole method with a sensitivity of 0.05-0.07 ng/ml. The values measured in the ewes and lambs in our experiment are similar to those reported by Eliot *et al.* (1981) in the maternal and foetal plasmas of sheep during the hours before spontaneous parturition ; these authors compared CA concentrations in maternal and foetal plasmas during spontaneous or cortisol-induced parturition. Our values are also in good agreement with those found by Ben-Jonathan *et al.* (1983) in pregnant ewes and their foetuses between days 125 and 140 of gestation. However, our results demonstrate that 24 h before spontaneous parturition, foetal plasma CA concentrations were lower than those measured in the same animals at birth ($P < 0.01$) and 2 h ($P < 0.01$), 24 h ($P < 0.05$) and 48 h ($P < 0.05$) later. This increase in foetal plasma CA concentration during labour did not seem to depend on increased placental CA transfer from the dam to the foetus. CA do not easily cross the placenta in humans (Saarikoski, 1974), ovines (Jones and Robinson, 1975) or other species (Anton, 1979). Furthermore, no significant increase in maternal plasma A, NA or DA concentrations was seen in our animals during the experimental period (fig. 1). Comline and Silver (1961) studied the influence of asphyxia (induced by ligation of the umbilical cord) on the release of A and NA by the ovine foetal adrenal medulla from 80 days of gestation until term. They demonstrated that from relatively early in pregnancy (80-90 days), the adrenal medulla appears to react directly to asphyxia by secreting a high proportion of NA. About 15-20 days before term, a nervous component was demonstrated which depended on the integrity of the splanchnic nerves and contained large amounts of both A and NA. At birth, the adrenal of the lamb contained a high amount of A, the A concentration gradually increasing during the last month *in utero.* This would explain the observed increase in plasma NA and A concentrations at parturition in our lambs (fig. 2). The slight simultaneous decrease in blood pH from 7.35 ± 0.02 before birth to 7.29 ± 0.03 immediately after birth ($P < 0.05$) might also indicate that the transient hypoxia associated with delivery would also stimulate A and NA release (Comline, Silver and Silver, 1965 ; Cohen, Piasecki and Jackson, 1982). Hypothermia would act synergistically (Eales and

Small, 1980). Such an increase in plasma NA at birth, stimulating triidothyronine production in brown adipose tissue by the process of thyroxine monodeiodination (Silva and Larsen, 1983), might be partly responsible for the increase in plasma triiodothyronine concentration observed at birth in these lambs (Davicco, Lefaivre and Barlet, 1982). This does not exclude the possibility that the increase in plasma CA concentration at birth might partly result from an increase in CA's of other origins than the adrenals (Roffi and Motelica-Heino, 1975 ; Padbury *et al.,* 1981).

During the first 10 days of postnatal life, calves delivered by caesarian section before term are characterized by higher plasma DA and NA concentrations than those measured in calves born spontaneously at term (fig. 2). In rats stress such as immobilization or electric footshocks induces a sharp and quick increase in both adrenal and plasma DA concentrations. The conversion of DA to NA and NA release each account for approximately one-half of the DA that disappears from the adrenals during strong sympathetic stimulation in rats (Snider and Kuchel, 1983). Such a stimulation probably occurred in the calves delivered by caesarian section before term (fig. 2). After the umbilical cord of lambs delivered by caesarian section at term or before, was cut, there was a marked increase in circulating NA and A. However, the preterm animals exhibited a delayed but exaggerated elevation of both NA and A relative to term lambs (Padbury *et al.,* 1985).

Mean plasma A (114 ± 19 pg/ml) and NA (200 ± 60 pg/ml) concentrations measured in calves born spontaneously at term were not different from those measured in 1 to 3-month old calves (A : 100 pg/ml ; NA : 200 pg/ml) (Blum *et al.,* 1982). However, plasma DA concentration measured in our animals (380 ± 57 pg/ml) was higher than that reported by Blum *et al.* (1982). This might be due to the fact that our animals were younger than theirs. L-hydroxyphenylalanine (dopa, the precursor of DA formation) concentration is very high in ovine foetal plasma (Ben-Jonathan *et al.,* 1983). This would also explain why plasma DA concentration in calves delivered before term remained higher until day 10 than in those born spontaneously at term (fig. 2). No significant variation in plasma A concentration was observed in either group of calves ; similarly, in Jersey calves no significant change in plasma A level was observed until 15-20 days after birth (Silver, 1960).

No calf born spontaneously before term was available so that our results did not permit us to determine the putative influence of caesarian section on plasma CA concentration. In newborn human babies considerably higher plasma CA levels were found after instrument delivery than after normal labour. These levels were very low after caesarian section under general anaesthesia (Hägnevik *et al.,* 1984).

These results, together with those previously obtained on glucocorticoid secretion in preterm calves (Richet *et al.,* 1985), indicate that both the cortex and the medulla of the adrenal glands continue their development after birth in calves delivered by caesarian section before term. This might partly explain the decreased resistance of these animals to various stresses occurring during the period of adaptation to extrauterine life.

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Résumé. *Catécholamines plasmatiques chez l'agneau et le veau au cours de la période périnatale.*

Les catécholamines (CA) plasmatiques (adrénaline, A ; noradrénaline, NA ; dopamine, DA) ont été dosées par radioenzymologie chez 7 brebis et leurs 7 fœtus (porteurs de cathéters carotidiens chroniques *in utero*) au cours des 19 derniers jours de gestation (127-145) et pendant la première semaine postnatale. Elles ont été aussi mesurées chez 18 veaux Holstein-Frisons au cours du premier mois postnatal. Neuf veaux étaient nés spontanément à terme (278 j). Les 9 autres avaient été délivrés par césarienne au 260e jour de gestation.

Chez les brebis, les CA plasmatiques n'ont pas présenté de variation significative pendant la période expérimentale. Chez les agneaux, au moment de la délivrance, les concentrations plasmatiques en A, NA et DA s'élevaient jusqu'à 1 800 $\pm$ 520 pg/ml, 3 782 $\pm$ 781 pg/ml et 575 $\pm$ 90 pg/ml, respectivement. Elles restaient supérieures aux concentrations mesurées chez les brebis jusqu'à 2 h après la naissance pour l'adrénaline et jusqu'à 48 h après la naissance pour la noradrénaline et la dopamine.

Chez les veaux nés par césarienne, au 5e et au 10e j après la naissance, les concentrations plasmatiques en NA et en DA étaient supérieures à celles mesurées aux mêmes âges chez les veaux nés spontanément à terme. L'adrénaline n'était jamais différente chez les 2 lots de veaux.

Ces résultats paraissent indiquer que la décharge de CA survenant normalement au moment de la délivrance chez les animaux nés spontanément à terme apparaît plus tardivement chez ceux délivrés par césarienne. Cette immaturité de la médullosurrénale, associée à celle précédemment démontrée de l'axe hypophyso-corticosurrénalien chez ces mêmes animaux (Richet *et al.*, 1985), peut expliquer leur moins grande résistance aux différents stress survenant au cours de la période d'adaptation à la vie extrautérine.

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