Influence of experimental acidosis on the concentrations of thyreostimulin (TSH) and iodothyronines (total T4, free T4, T3) in the plasma of the newborn lamb

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Reprod. Nutr. Dev. (1989) 29, 509-515

Original article

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(received 7 December 1988, accepted 4 September 1989)

Summary — The effects of acute acidosis on neonatal thyroid function were studied by infusing HCl for 4 h in 42 to 54-h-old lambs. Animals of the same age, used as controls, were simultaneously infused with physiological saline. HCl infusion induced a sharp decrease in blood pH and total restoration did not occur before 48 h. When compared to control lambs, this experimental acidosis was associated with slight, but significant, decreases in plasma TSH, total T4, free T4 and total T3 levels, and in values of the free T4/total T4 ratio; the T3/FT4 ratio was not affected. The values of RT3/FT4 ratio were significantly increased in acidotic lambs. It is concluded that acidosis induced only modest secretory changes in neonatal thyroid function and slightly reduced the proportion and the amount of free T4.

lamb — newborn — thyroid — TSH — T4 — free T4 — acidosis

Résumé — Influence d'une acidose expérimentale sur les concentrations plasmatiques de thyréostimuline (TSH) et d'iodothyronines (T4 totale, T4 libre, T3) chez l'agneau nouveau-né. *Les effets d'une acidose expérimentale sur la fonction thyroïdienne néonatale ont été étudiés en perfusant de l'acide chlorhydrique pendant 4 h à des agneaux âgés de 42 à 54 h. Des animaux du même âge, utilisés comme témoins, ont été simultanément perfusés avec du sérum physiologique. La perfusion d'HCl a induit une diminution rapide du pH sanguin, le retour à la valeur initiale nécessitant 48 h. Par comparaison avec les évolutions observées chez les agneaux témoins, cette acidose expérimentale est associée à des diminutions modérées, mais significatives, des concentrations plasmatiques de TSH, T3, T4 libre et totale, et de la valeur du rapport T4 libre/T4 totale; la valeur du rapport T3/T4 libre n'est pas modifiée. Par contre, l'acidose induit une élévation importante de la valeur du rapport RT3/FT4. En conclusion, une acidose induit par elle-même des modifications sécrétoires très modérées sur la fonction thyroïdienne néonatale et réduit légèrement mais significativement la proportion et la quantité de T4 libre circulante.*

agneau — nouveau-né — thyroïde — TSH — T4 — T4 libre — acidose

INTRODUCTION

We have previously shown in the lamb, that the neonatal rise in free T4 (FT4) levels was more important and that its duration was longer than the increase in plasma total T4 levels (Cabello, 1987; Cabello & Wrutniak, 1986, 1988). As FT4 is the direct substrate for cell triiodothyronine (T3) production, such a phenomenon is probably important for the establishement of neonatal thyroid function. It could be particularly involved in the persistancy of elevated plasma T3 concentrations after the neonatal T3 surge, despite a significant decrease in plasma total T4 levels.

At birth, spontaneous acidosis, probably due to the birth stress, generally occurs and is followed by a progressive normalization. We have previously observed that the neonatal rise in blood pH has a strong parallelism with the increase in the value of the plasma FT4/T4 ratio occurring during the first 16 h of life (Cabello, 1987). This suggests that there is some influence of blood pH on the affinity and/or capacity of carrier proteins for T4, and therefore, on FT4 concentrations.

In lambs, as in calves, dystocial births, associated with acute asphyxia and acidosis, strongly reduced plasma total T4 and T3 levels (Cabello & Wrutniak, 1984; Vermorel *et al.,* 1984). In addition, Klein *et al.* (1979) also reported, in lambs, that asphyxia induced a significant decrease in plasma thyreostimulin (TSH), T4 and T3 concentrations.

Therefore, we studied the influence of an experimental acidosis on thyroid function in newborn lambs in order to assess if the spontaneous neonatal acidosis and the following restoration of blood pH contribute to the above changes in plasma free T4 levels occurring after birth.

MATERIALS AND METHODS

Sixteen 42—54-h-old Limousin x Romanov lambs, spontaneously born at the expected term of the gestation, were used in this experiment. This age was chosen in order to avoid the large postnatal increases in plasma T4, T3 and TSH levels, the major part of the subsequent decrease in T4 and TSH concentrations, and also the spontaneous changes in blood pH. All animals were catheterized at 09.00 h as follows : the left jugular vein was punctured with a sterilized needle (inner diameter, 0.8 mm); a sterile polythene catheter (Biotrol No. 1); inner diameter, 0.3 mm; Biotrol Laboratory, Paris) was inserted into the jugular vein through the needle, which was then removed. The catheter, maintained by adhesive ribbons, was filled with sterile heparinized physiological saline and stopped with an aseptized plastic stopper. Control animals ($n = 8$; birthweight : 3.67 ± 0.16 kg) were infused at 14.00 h with physiological saline (5 ml/h) for 4 h. As previously described (Bureau & Begin, 1982), at the same time, acidosis was induced in 8 treated lambs (birthweight : 3.58 ± 0.13 kg, NS) by infusion of a HCl 4 mmol/h/kg solution (5 ml/h) until blood pH decreased to a value < 7.25 (1.7 ± 0.1 h); thereafter, HCl was infused at a reduced rate (2 mmol/h/kg, 5 ml/h) to reach a total infusion time of 4 h. Before and after infusion, all animals were allowed to suckle their mothers *ad libitum*; 2 h after the onset of infusion, they were bottle-fed with 100 ml artificial milk, in order to avoid TSH, T4 and T3 changes induced by underfeeding (Wrutniak & Cabello, 1987).

Blood samples (2 ml) were collected, avoiding contact with air for blood pH measurement, from the right jugular vein just before the onset of infusion, and 30 min, 1, 2, 3, 4, 6, 8, 12, 24 and 48 h after. Plasma was separated within 15 min by centrifugation and kept frozen at – 20 °C until analysis. Additional 500-μl blood samples were collected every 30 min during infusion, in order to control the blood pH decrease.

Plasma hormonal concentrations (total T4, free T4, total T3, RT3, TSH) were measured by radioimmunoassay as previously described (Cabello & Levieux, 1980; Wrutniak *et al.,* 1985, 1987). Total T4 and T3 were assayed using the T4K and T3K kits purchased from CEA (France); the sensitivities of the methods

were respectively 1 ng and 150 pg/ml plasma, and the reproducibilities were 2.5 and 3.5%. RT3 levels were measured with reagents furnished by Dr. Guennec (CEA, France); a rabbit RT3 antibody was used, and free and antibody-bound RT3 were separated with polyethylene glycol after addition of 50 µl equine serum; the sensitivity of the assay was 100 pg/ml and the reproducibility was 3%. FT4 levels were measured with the Lisophase technique of Lepetit Laboratory (provided by CEA, France), including a separation of free and carrier-bound T4 by Sephadex LH2O chromatography; the reproducibility of the assay was 4% and the sensitivity was 0.8 pg/ml. Ovine TSH was measured using a double antibody technic, with TSH and TSH-antibody supplied by the National Hormone Program (NIADDK, Bethesda); the sensitivity of the assay was 0.05 ng/ml and the reproducibility was 8%. Blood pH was measured extemporaneously with an accurate Metrohm blood pH meter (0.001 pH unit).

The mean ± SEM was calculated at each stage for each hormone. As values of the FT4/T4 ratio evidenced a log-Gaussian distribution, they were converted to the logarithm form, and were given after decimal conversion of the mean ± SEM of the logarithms. Within-groups differences were tested by the paired Student's *t*-test; differences calculated between the values, measured at the start and at the end of the infusion for each parameter, were compared between groups by the Mann-Whitney U-test.

RESULTS (Fig. 1)

In control lambs, blood pH rose slightly during the first 30 min of infusion from 7.41 ± 0.01 to 7.44 ± 0.01 ($P < 0.01$) and did not change thereafter. In treated animals, it decreased sharply during HCl infusion from 7.41 ± 0.01 to 7.10 ± 0.01 ($P < 0.001$); 30 min after the onset of infusion, it was significantly lower than the initial value ($P < 0.001$). Then it rose progressively after HCl withdrawal; however, a value similar to that recorded in controls was not reached before 48 h post-infusion.

Although not significant, a decrease in plasma TSH levels could be observed in control animals throughout the experimental period. In treated lambs, they decreased throughout the infusion period from 0.31 ± 0.05 to 0.22 ± 0.04 ng/ml ($P < 0.01$); this decrease was significantly greater in HCl than in control lambs (U-test, $P < 0.1$). Thereafter, they progressively rose to reach the post-infusion value 48 h after the infusion onset (0.29 ± 0.05 ng/ml, $P < 0.005$).

Plasma total T4 levels did not significantly change during the infusion period in control lambs, but significantly decreased in HCl treated animals from 10.0 ± 0.9 to 8.9 ± 0.9 µg/dl ($P < 0.025$). In the two groups, they remained unchanged from the end of infusion to time 12 h, and significantly declined until 48 h (controls, $P < 0.005$; HCl, $P < 0.001$).

Whereas plasma-free T4 levels significantly decreased during the infusion period in treated animals from 43.5 ± 4.5 to 32.5 ± 2.5 pg/ml ($P < 0.005$), they did not significantly change in control lambs; when tested by the U-test the differences between the initial and final FT4 values were significantly higher in HCl animals ($P < 0.05$). Throughout the observation period, they decreased progressively in the two experimental groups (controls, $P < 0.05$; HCl, $P < 0.005$).

During HCl infusion, whereas, the values of the FT4/T4 ratio significantly declined in treated lambs from 43 (decimal conversion of the mean ± SEM of the logarithms : 40—46) to 37 (34—40) ($P < 0.01$), they did not change in control animals : when tested by the U-test, the differences between the initial and final values were significantly higher in HCl animals ($P < 0.05$). Throughout the

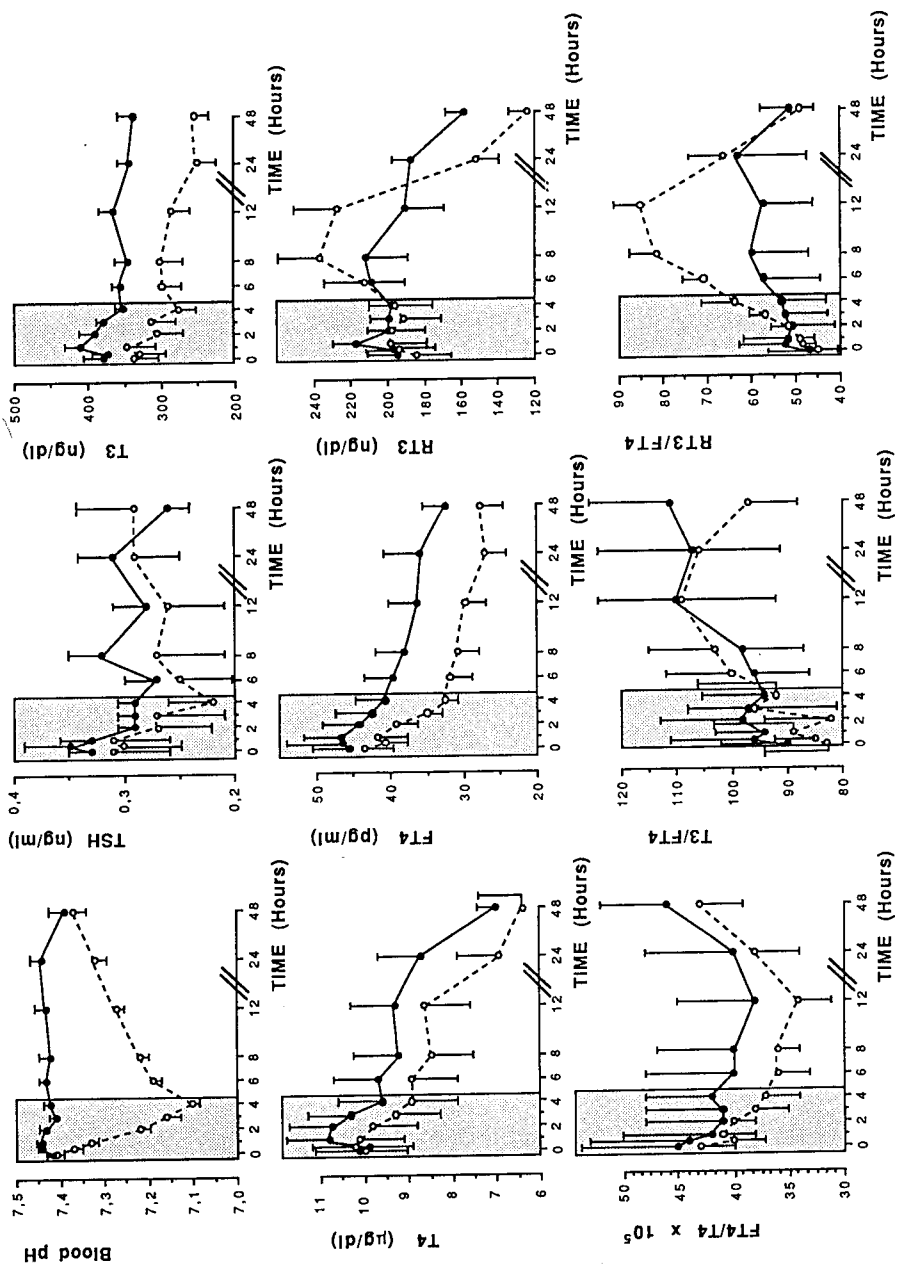

Fig. 1. Changes in blood pH, values of the FT4/ T4 and T3/FT4 ratios, and plasma concentrations of TSH, total T4, free T4 and T3 in physiological saline (•—•), or HCl infused lambs (o—o). Mean ± SEM; ▒ Infusion period.

observation period, they progressively decreased from time 0 to time 12 h in the two groups (controls, $P < 0.05$; HCl, $P < 0.01$) and rose until 48 h (respectively, $P < 0.025$ and $P < 0.001$).

In control lambs, plasma T3 concentrations did not significantly change during infusion or the total observation period. During HCl infusion, they significantly decreased from 335 ± 33 to 277 ± 27 ng/dl ($P < 0.05$) and remained unchanged thereafter. No significant changes have been recorded in the values of the T3/FT4 ratio for the two groups of animals.

During HCl infusion, plasma RT3 concentrations did not change in the two groups of lambs; however, they significantly increased ($P < 0.01$) between 4 and 8 h in treated animals and fell thereafter ($P < 0.001$). In control animals, they remained unchanged until 8 h and decreased thereafter ($P < 0.01$).

The value of the RT3/FT4 ratio did not change throughout the infusion period in control lambs, whereas, a sharp rise was recorded in treated animals during HCl infusion ($P \leq 0.001$) and the following 6 h ($P < 0.001$); they decreased to the post-infusion value between 12 and 48 h ($P < 0.001$). When tested by the U-test, the changes occurring during the infusion period, or the first 6 h, were significantly different in the two groups of lambs ($P < 0.001$).

DISCUSSION

In control lambs, plasma total and free T4 concentrations, and also TSH levels (not significantly), slightly decreased throughout the experimental period without changes in T3 levels. These results are in agreement with the physiological changes in the concentrations of these hormones previously reported from 16 to at least 48 h *post partum* in similarly reared lambs (Cabello, 1987; Cabello & Wrutniak, 1988) and showing a progressive decrease following the neonatal surges.

Using the experimental schedule previously proposed by Bureau & Begin (1982), we induced severe acidosis in 24-h-old lambs. At birth, the lowest blood pH observed in 42 lambs was 7.15 (Cabello, 1987), a value higher than that reached in our experiment (7.10 ± 0.01). However, similar values have been reported at birth in calves classified as acidotic (Demigné & Remesy, 1984), and pH normalization required the same time as that shown in the present work. Therefore, these aspects did not differ for HCl or lactic acidosis (spontaneous neonatal acidosis).

In a previous work (Cabello & Wrutniak, 1984), we reported that dystocial births, associated with acute asphyxia and acidosis, markedly reduced plasma total T4 and T3 levels in newborn lambs to levels which were sometimes undetectable; the same observation was published in newborn calves by Vermorel *et al.* (1984). Klein *et al.* (1979) also reported that asphyxia in the lamb induced a significant decrease in plasma TSH, T4 and T3 levels. In contradiction to these results, Dos Santos (1987) observed that hypoxia, induced by prolonged inhalation of a 91.5% nitrogen — 8.5% oxygen mixture at birth, induced a sharp decrease in blood pH, in lambs as in calves, without affecting plasma T4 and T3 levels. However, dystocia or hypoxia induces probably more complex changes than acidosis alone.

Our results indicate that acidosis *per se* induces only moderate changes in plasma thyroid hormone and TSH levels, and therefore, that the changes observed in dystocia or hypoxia are probably more

related to other consequences of oxygen deficiency. As TSH levels showed the same pattern as that of blood pH (decrease during HCl infusion followed by a progressive restoration), the major result recorded in this work is that blood pH affects pituitary TSH secretion. Therefore, changes in plasma T3 and total T4 levels are probably a consequence of this alteration in pituitary TSH secretion. The rise in RT3 concentrations observed during the 4 h following the end of HCl infusion needs some clarification; however, as this iodothyronine has essentially an extrathyroidal origin (Chopra *et al.,* 1975), this observation could be explained by acidosis-induced changes in RT3 clearance and/or T4-5-deiodinations.

Lastly, it appears that a decrease in blood pH could slightly reduce the proportion and the amount of circulating free T4, probably by affecting the affinity of carrier proteins for T4; however, as these levels were not clearly influenced by the progressive restoration of blood pH, the physiological rise in blood pH occurring over the first 16 h of life is probably not the major factor involved in the increasing FT4/T4 ratio recorded after birth (Cabello, 1987; Cabello & Wrutniak, 1986, 1988).

ACKNOWLEDGMENTS

We thank Mrs C. Foucher and Mr P. Chagnaud, R. Bardoux and R. Dabert for their excellent technical assistance. We are grateful to the National Institute of Arthritis and Metabolic Diseases, National Pituitary Agency, for the gift of ovine TSH and ovine TSH antisera.

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