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Fetal hypophysis as the main source of serum TSH in fetal rat

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Summary. Decapitation performed at days 17–18 leads to a drastic drop (82%) in blood TSH of 19 and 21-day-old rat fetuses below the mother's level. ^{125}I-TSH injected at 21 days into the mother's bloodstream is not found in fetal blood. The fetal hypophysis is the main source of fetal plasmatic TSH.
Key words. Rat fetal hypophysis; placental transfer.

TSH has been measured in the plasma of newborn rats and in 18–21-day-old fetuses[1–3]. Though it is generally accepted that the fetal thyroid pituitary axis develops independently of maternal TSH, the fetal hypophysis was not actually demonstrated to be the main source of the fetal serum TSH. In the literature, a few reports give indirect evidence of the probable lack of TSH transfer from the maternal blood to the fetus; propylthiouracil given to pregnant rats[4,5] or rabbit[6] induces a hypertrophy of the fetal thyroid in normal but not in decapitated fetuses; this indicates that maternal TSH does not replace fetal hormone for the fetal goiter induction. The injection of TSH into pregnant rats enhances formation of colloid droplets in the thyroid of the mother but not in that of the fetus, whereas increased fetal serum TSH stimulates the appearance of colloid droplets in the fetal thyroid[7]. To our knowledge, transplacental permeation of radioactive-labeled TSH has been studied only in sheep[8,9], where no transfer was found in either direction, i.e. mother to fetus or fetus to mother. However it cannot be automatically assumed that such a transfer does not occur in the rat because of the hemochorial placenta of this species; only 3 tissue layers separate the maternal and fetal circulations, instead of 6 layers in the epitheliochorial sheep placenta[10]. TSH might permeate the rat placenta more easily than the sheep placenta. This study was designed in an attempt to trace the origin of the fetal TSH in the rat; firstly, the effects of the suppression of the activity of the fetal hypophysis by decapitation on blood TSH level, and secondly, the transfer, to the fetus, of ^{125}I-TSH injected in the mother were studied.

Material and methods. Pregnant Wistar rats purchased from a breeding center (IFFA-CREDO, l'Arbresle, 69210 France) were used in these experiments. The mating day, determined by the presence of spermatozoa in the vaginal smear, was regarded as day 0 of gestation. On their arrival at the laboratory, the animals were housed at 23 °C and fed ad libitum on UAR commercial diet, weekly supplemented with salad and bread.

During all surgical procedures mothers were anesthetized with ether. In some litters 3 or 4 fetuses were totally decapitated at day 17 or 18 (section at the neck level) according to the technique of Jost[11–12]. At 19 or 21 days blood samples were taken from the mother and from both decapitated and unoperated fetuses. Utero-placental circulation was not interrupted during sampling[13]. Samples were collected on EDTA and centrifuged at 0–4 °C. The plasma fraction was immediately frozen pending assay. Operated animals were separately caged in a quiet room.

Plasma TSH was measured using the radioimmunoassay reagents of the NIADDK rat pituitary program. TSH iodination was achieved using ^{125}I (Amersham, England) and iodogen (Pierce, Chem. Co., USA) according to the technique of Salicinski et al.[13]. Results are expressed as NIAMDD-r-TSH-RP1. The detection limit of the assay was 6 ng/ml; the percentage of recovery of added amounts of TSH was 103 ± 2%.

In another experiment, 10^7 to 1.5×10^7 cpm of ^{125}I- TSH solution were injected at day 21 into the blood of the anesthetized mother via the carotid. At various times between 15 and 180 min, blood samples were withdrawn from the mother's carotid and from 2 or 3 fetuses. The total radioactivity of the samples including both ^{125}I-TSH and iodine resulting from TSH catabolism was measured. The ^{125} I-TSH fraction was ascertained by recognition with an anti-TSH-antiserum (NIAMDDDK anti-TSH-S5) and by immune complex precipitation with a second antibody (preliminary experiments were carried out to determine the amount of antibodies required to precipitate 80% of a 10^5 to 1.5×10^5 cpm aliquot of TSH solution). The animals were quickly killed at the end of the blood sampling before recovery from the anesthesia.

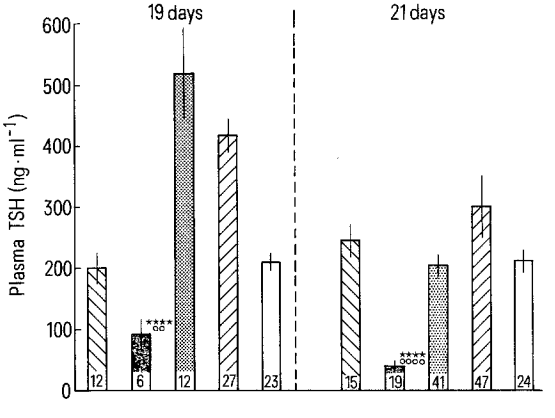

Figure 1. Plasma TSH at day 19 and 21 in normal rat fetuses, their unoperated mothers, in fetuses decapitated at days 17 or 18, unoperated littermate fetuses, and laparotomized mothers. The number of measurements is indicated at the foot of each column. Standard deviations are represented by vertical bars. Statistical comparison was made between decapitated fetuses and their mothers: ○○, $p < 0.02$; ○○○○, $p < 0.001$ and between decapitated fetuses and their unoperated littermates: ****, $p < 0.001$.

Results. Figure 1 shows the effects of surgical opertion of the fetus, performed on days 17 or 18, on plasma TSH levels at 19 or 21 days. After 2–4 days, the laparotomy does not significantly modify the thyrotropin concentrations either in the pregnant rat nor in the untouched fetuses, compared with the values observed in unoperated animals. In the decapitated fetuses, plasma TSH is 82% lower than in their unoperated littermates. A small but statistically significant (different from 0) quantity of an immmunologically TSH-like substance is measurable in the blood of the decapitated fetuses. With regard to the concentration of this 'residual TSH', it is interesting to note that it is significantly higher at day 19 than day 21 (< 0.01) and that it is significantly lower than maternal plasma TSH ($p < 0.002$ and $p < 0.001$ at 19 and 21 days respectively).

The second figure describes the changes in the levels of total serum ^{125} I and immunoprecipitable ^{125} I-TSH in mothers and 21-day-old fetuses in the 3-h-period following injection of the radioactive hormone into the mother. In the mother, total radioactivity drops rapidly and exponentially during this period and immunoprecipitable ^{125} I-TSH exhibits a similar evolution although the curve is somewhat below that of the total radioactivity. In the fetuses, total ^{125} I radioactivity shows a slow rise but stays much lower than in the mother. The immunoprecipitable ^{125} I-TSH in fetuses in almost totally indiscernible above the background within 3 h after injection.

Discussion. The TSH plasma levels found in mothers and fetuses are clearly higher than the limit of sensitivity in the TSH assay used. In 21-day-old fetuses and neonates, serum TSH has previously been observed to be lower than in 18–20-day-old fetuses and higher than in the mother[1–3]. Since the rat fetal hypophysis produces TSH[15,16] and since the removal of the brain, the pituitary being left in situ (encephalectomy) does not depress the TSH level in the fetal plasma[17], the 82% drop in serum TSH in decapitated fetuses (fig. 1) is evidence that the pituitary is the greatest source of TSH in the fetal rat. Moreover the plasma TSH level of decapitated fetuses remains much lower than that of the mother in the 2-4 days following the operation, which would indicate that even if there is some TSH transfer from the mother it is insufficient to restore the fetal level of thyrotropin. The second figure shows that ^{125}I-labeled-TSH injected into the mother is not transferred to the fetus in an amount detectable with the technique used. An important remark must be made; from these results, the biological half life of radiolabeled TSH in the mother's circulation is 35 min. This is a somewhat longer time than the 25 min found in euthyroid male rats[18], but is in the range of the half life in hypothyroid or triiodothyronine injected rats[18] or in pregnant ewes[8]. The slower rate of the ^{125}I-TSH degradation in the mother may originate from special features of the female and pregnancy physiology; the TSH metabolic clearance rate tended to the higher in men that in women, but this sex difference is strongly reduced when corrected for body area[19]. The modification of the TSH molecule by iodination, although this does not impair the recognition by antibodies, may also have induced this decrease of TSH catabolism.

The small quantity of immunologically TSH-like substance found in the plasma of decapitated fetuses cannot result from a transfer of TSH from mother to fetus: the second figure gives direct evidence that TSH injected into the mother rat does not reach the fetal blood stream.

The 'residual TSH' could be veritable TSH left over from before decapitation; the half life of TSH in the rat fetus is not known, but if it is comparable to that of the fetal sheep (i.e. 47 min)[8] or of the adult rat[18] then TSH would have disappeared by 2 or 4 days after decapitation. Another hypothesis would be that this substance, genuine or facsimile immunological TSH, is secreted in fetal blood by a non-cephalic organ (the placenta for example). A thyroid-stimulating glycoprotein has been extracted from the human placenta[20–22]. Human chorionic gonadotropin also exhibits biological[23–26] and immunological[27] properties similar to TSH. But the luteotropic and lactogenic activities of the rat placenta during the second half of pregnancy are well established[28,29]. The concentration of gonadotrophin in the placenta[30], and of the placental lactogen in the serum[31] present two peaks, the second at day 19. The TSH-like substance found in the decapitated fetus is also significantly higher at this stage (fig. 1). As the rat placental hormones are not available in a purified form it is not known whether they cross-react with the anti-TSH antibodies. It is also not known whether the 'residual TSH' found in the decapitated fetus stimulates the thyroid or if it has gonadotropic or lactogenic activity. Other tissues than placenta may also be able to secrete TSH-like substances; human lymphocytes stimulated by mitogen may produce immuno-reactive thyrotropin in vitro[32].

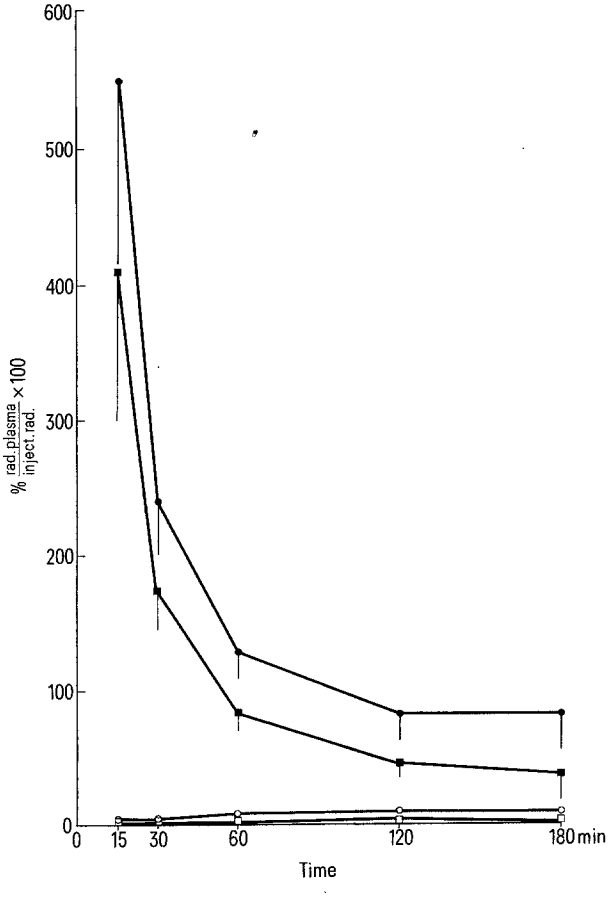

Figure 2. Changes in levels of total ^{125} I radioactivity (dots) and immunoprecipitable ^{125} I-TSH (squares) in mothers (dark symbols) and fetuses (open symbols) during the 3-h-period following injection of ^{125} I into the mother's bloodstream. Concentrations are expressed as 100 times the percentage of injected radioactivity. Each point is the mean of 5 experiments. The fetuses were 21 days old.

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Two sulfur-containing ansamycin antibiotics from *Streptomyces albolongus*[1]

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Summary. Two sulfur-containing ansamycin antibiotics were isolated from the culture broth of *Streptomyces albolongus* C-46366; the major one was identical with awamycin and the minor one was a new ansamycin antibiotic, ansathiazin. Their structures were elucidated from their reactions and spectroscopic analyses. These antibiotics were active against gram-positive bacteria, acid-fast bacteria and a protozoan.
Key words. Ansamycin; ansathiazin; awamycin; *Streptomyces albolongus* C-46366; gram-positive bacteria.

In our search for ansamycin antibiotics using a rifampicin-resistant mutant of *Staphylococcus aureus* FDA 209P as an indicator, two sulfur-containing ansamycin antibiotics, awamycin[2] (**1**) and ansathiazin (**2**) (fig. 1), were isolated from the culture filtrate of *Streptomyces albolongus* C-46366, which was obtained from a soil sample collected in Okinawa, Japan.

Taxonomical studies showed that the strain C-46366 was assigned to chemotype I and to the white (W), rectiflexibiles (RF), chromogenic (C^+) and glabrous (SM) group within the genus *Streptomyces*. By other taxonomical characteristics, the strain C-46366 was judged to be related to *S. albolongus*[3] (from the Bergey's Manual of Determinative Bacteriology 8th edn). Strain C-46366 was subsequently compared with *S. albolongus* IFO 13465 (ISP 5570) under the same cultural conditions, and no marked differences were found. Thus, the strain was named *S. albolongus* C-46366. A scanning electron micrograph of the strain is shown in figure 2. The strain was clearly different from *Streptomyces* sp. No. 80–217, the awamycin-producing actinomycete[2], at the following points: spore chain morphology, spore surfaces and aerial mass color.

Table 1. Physicochemical properties of **1** and **2**

	1	**2**
m.p.	162–165 °C	170–175 °C (dec)
$[\alpha]_D$[a]	+949° (c 0.17)	−32° (c 0.1)
EI-MS	743 (M^+)	745 (M^+)
SI-MS	745 $(M+3)^+$	768 $(M+Na)^+$
Analysis found	C, 60.66; H, 6.69; N, 1.89; S, 4.11 (%)	C, 59.86; H, 6.48; N, 1.98; S, 3.52 (%)
Calculated	C, 60.62; H, 6.69; N, 1.86; S, 4,26 (%)	C, 59.58; H, 6.35; N, 1.88; S, 4.29 (%)
Formula	$C_{38}H_{49}No_{12}S.1/2H_2O$	$C_{37}H_{47}NO_{13}S$
UV: λ_{max}nm (ε) in MeOH	218 (40,200) 443 (4,890)	210(32,900) 273 (23,500) 420 (4,700)
IR: ν_{max} cm^{-1} in KBr	3450, 2980, 1730, 1670, 1630, 1470, 1410, 1380, 1300, 1260, 1210, 1170, 990	3450, 2980, 1720, 1670, 1640, 1600, 1460, 1410, 1380, 1290, 1150, 1100, 990
TLC(SiO_2)	Rf 0.57 ($CHCl_3$:MeOH = 9:1) Rf 0.25 (CH_2Cl_2:MeOH = 25:1)	Rf 0.37 (the same) Rf 0.10 (the same)
HPLC (ODS)	Rt 4.7 min (CH_3CN:H_2O = 7:3)	Rt 5.1 min (the same)

[a]The specific rotations of all samples herein were measured at 22–25 °CX in $CHCl_3$.