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MAMMOTROPHIC AND GROWTH PROMOTING ACTIVITIES OF A PLACENTAL HORMONE IN SHEEP

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SUMMARY

An ovine placental lactogenic hormone has been isolated and purified. This placental hormone proved to be able to ensure mammary gland growth in pregnant ewes injected with ergocryptin. We show here: 1. The lactogenic activity of this hormone by using cortisol as inductor of milk secretion during pregnancy in sheep in which prolactin has been suppressed by ergocryptin treatment. 2. The growth promoting activity by injecting the purified hormone in hypophysectomized rats. 3. The half-life and the pattern of change of prolactin-like and growth hormone-like activities during pregnancy in sheep by their respective radioreceptor assays. Detection of these two activities in fetuses suggests a possible role of this hormone in the fetal growth. All these facts confirm the existence in sheep of a lactogenic placental hormone exhibiting a marked growth hormone activity. Ovine chorionic somatomammotrophin seems therefore a suitable name.

Placental lactogenic and growth hormone activities have already been found in primates. An ovine placental lactogenic hormone (OPL) was purified and characterized [1–4]. Mammogenic activities of OPL were demonstrated *in vivo* in pregnant ewes depressed in prolactin (bromoergocryptin treatment). Studies of mammary tissues obtained from days 80–140 of pregnancy indicated that mammary gland dry weight and DNA contents were normal in bromocryptin treated ewes [5].

Lactogenic activity of OPL was demonstrated *in vivo* by experimental induction of milk secretion during pregnancy in sheep (Fig. 1). Milk secretion induced by cortisol (25 mg/animal, twice daily) in pregnant ewes or in aprolactinemic pregnant ewes (2 bromo-α-ergocryptin treatment, CB 154 Sandoz, 1 mg/animal, twice daily) are of the same magnitude. One ewe aborted on day 125 of pregnancy showed a similar milk secretion and may be considered as a control of milk production at this stage of mammary growth.

In aprolactinemic and hysterectomized ewes, cortisol cannot induce milk secretion without lactogenic hormone and the very low level of prolactin in ewes injected with bromoergocryptin cannot induce milk secretion with cortisol.

In control pregnant ewes, teat stimulations cannot induce milk secretion without cortisol. These controls are consistent because all the ewes were milked twice daily throughout the experiments.

These experimental results show the lactogenic properties of OPL, *in vivo*. In pregnant ewes, OPL allows milk to be secreted by the mammary gland with cortisol and without prolactin.

Lactogenic activities of OPL were also studied *in vitro* with purified OPL. Lactogenesis was demonstrated by histological examination, by the appearance of lactose synthetase activities and casein synthesis in organ cultures of pseudopregnant rabbit mammary gland [6].

Thus, OPL appears to have the two properties of hypophyseal prolactin, *i.e.* mammogenic and lactogenic.

Growth promoting activities of OPL were shown with injections of purified hormone in hypophysectomized rats. Growth hormone activities were tested by

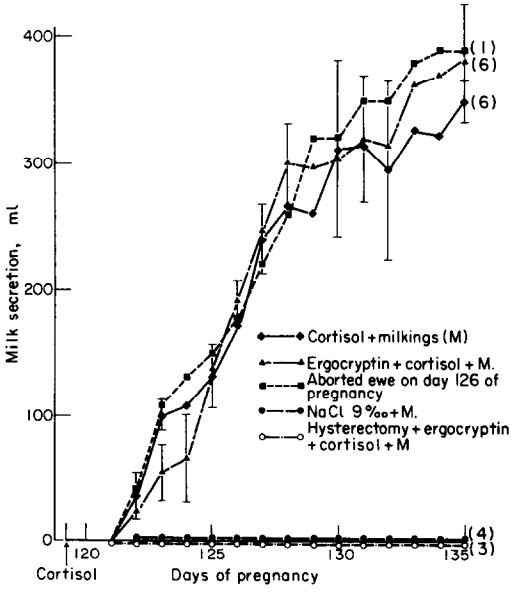

Fig. 1. Experimental induction of milk secretion during pregnancy in Sheep. ( ) number of animals, means ± S.E.M.

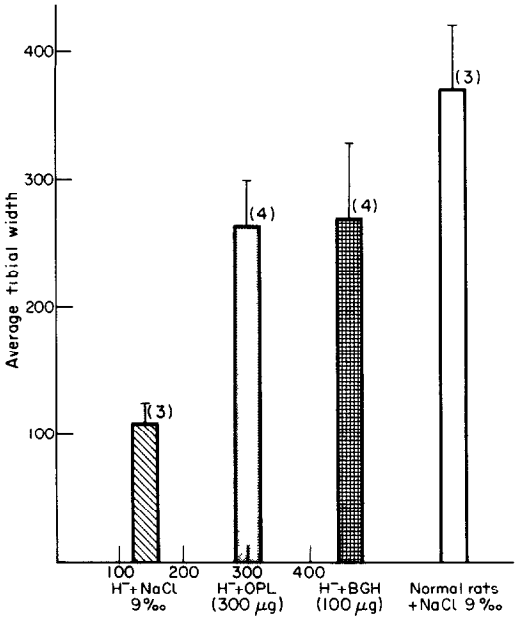

Fig. 2. Increase in epiphyseal cartilage plates in hypophysectomized rats (H^-) injected with OPL (or OCS) and BGH during three weeks. After hormonal injection during about two weeks, an antihormone effect was observed in body weight gain.

gain in body weight, increase in the width of the epiphyseal cartilage plate of the tibia (Fig. 2) and by radioreceptor assay on rabbit liver membranes with BGH Pentex standard (1 I.U./mg).

In control hypophysectomized rats, we have also injected purified OPL (300 μg prolactin equivalent) with antobodies against OGH (at a concentration able to neutralize about 200 μg of OGH) and we observed the same results with purified OPL alone.

These last facts prove that growth hormone activities of OPL cannot come from any foetal OGH contamination of OPL preparations.

Consequently, since OPL has mammotrophic and growth hormone activities, its name Ovine Chorionic Somatomammotrophin (OCS) appears suitable.

Pattern of change of prolactin-like activities and that of growth hormone-like activities during pregnancy were recorded in ovine blood by radioreceptor assays. Specific lactogenic or growth activities of OCS were determined by deduction of prolactin or growth hormone activities measured by radioimmunoassays [7, 8]. These levels were recorded in maternal and foetal blood. Serum ovine growth hormone-like activity seems to be about a third of prolactin-like activity.

So, the half-life of OGH-like activity appears to be nearly half of that of prolactin-like activity of OCS.

Maternal serum OCS pattern in sheep is comparable to that described in goat [9] (personal communication). It confirms the role of this hormone in mammogenesis and in lactogenesis.

Detection of GH activities of OCS in the foetus suggests that this hormone plays an important part in foetal growth.

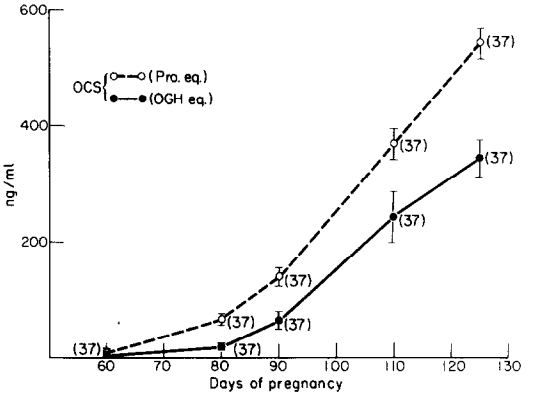

Fig. 3. Comparison between lactogenic and growth hormone activities in maternal blood from day 60 to 125 of pregnancy. This experiment was carried out on 37 pregnant ewes of the Préalpes du Sud breed.

The large growth hormone activities of OCS and its capacity to compete for HGH receptor sites [3] suggest a large structural homology between OCS and HGH. OCS might be useful to improve the knowledge about the biological active sites of these molecules.

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DISCUSSIONS

Thorburn. In the last two papers we have seen evidence of large amounts of ovine prolactin and ovine growth hormone in the fetal lamb and that they both seem to have growth promoting activities. But after birth, the levels of both these hormones decrease within the matter of a day or so and yet the lamb continues to grow at the same rate. I find that difficult to understand. I wondered if you have any ideas on that.

Martal. This is a difficult problem. During very early pregnancy OCS production occurs. Dr Friesen has determined plasma OCS in the mother's blood by radioimmunoassay at about days 30–40. We have determined in the placenta the OCS activity from day 18 of pregnancy onwards. In fetal blood, Bassett has determined OGH activity as soon as day 50 of pregnancy. So before this time, growth hormone activity seems to be due to OCS only. Boda has determined OGH in the fetal hypophysis from day 50 of pregnancy. We have studied the correlation curve between age of gestation and the cubic root of fetal weight. This line intercepts the age axis at about day 30. These facts may suggest a role of OCS in fetal growth. After this time both the fetal OGH and OCS may play a role during pregnancy. At birth we observe a drop of OCS levels as for fetal OGH, though lamb growth continues. In cattle, Trenkle has demonstrated a linear relationship between the serum GH concentration and the secretion rate/kilo/day and not between the GH level and the total weight. Hence it is necessary to consider the secretion rates, however we haven't yet all the facts to understand the phenomenon.

Friesen. I have no answers to Dr. Thorburn's question. I think really we are in an area here where more experiments are needed rather than more speculation. I would like to direct a question to Dr. Martal. We have noticed on your slide too that the ratio of prolactin to growth hormone-like activity in ovine serum samples measured by two receptor assays is in the neighbourhood of three to one. At times we have seen it as high as 10 to 1 in the serum, whereas in purified OPL preparations it is more like 1 to 1. I wondered what sort of differences you have observed when applying the two receptor assays either to your purified preparation or to your serum samples.

Martal. Our purified preparations of Placental Lactogen shows a ratio between the prolactin-like and growth hormone-like activity of about one or two and the maternal serum mean ratio is about three to one. Growth hormone activity per mg is nearly 0.3–0.5 I.U. in terms of radioreceptor or tibia-test with N.I.H. ovine growth hormone standard.

Solomon. I would like to ask Dr Lerner if he could tell us a little bit about how he studied prostaglandin synthesis, how he studied prostaglandin metabolism and what he is doing about the endoperoxides of the prostaglandin.

Lerner. We studied prostaglandin synthesis using a method we described in a publication last year (*Endocrinology* **97** (1975) 1071). In this method tritiated arachidonic acid is added to an incubation mixture containing the tissue homogenate in the presence of epinephrine and reduced glutathione. After the incubation period, the enzymatic reaction is stopped and the arachidonic acid and reaction products were extracted and identified through the use of thin-layer chromatography and liquid scintillation counting methods. Calculation is on the basis of pmol formed per h per mg protein.

In vitro prostaglandin metabolism methodology was also described in the above mentioned publication. This method involves the addition of tritiated prostaglandins and NAD^+ to the supernatant of centrifuged tissue homogenates, incubation of this mixture for varying periods of time (depending on the tissue), stopping the reaction, extraction, thin-layer chromatography, identification and counting the radioactivity with a liquid scintillator. The *in vivo* study of prostaglandin metabolism involves the injection of radioactive prostaglandin intravenously and then sacrificing the animals 10 s later and then processing the plasma from these animals as described above.

Solomon. What of metabolites?

Lerner. We determined the presence and percent of radioactivity of the 15-hydroxy, 15-keto, and 13, 14-dihydro keto prostaglandins as well as that of the non-metabolized prostaglandins but did not study the endoperoxides.

Crastes de Paulet. I would like to understand more precisely what exactly you did when you studied prostaglandin metabolism. Did you make incubations with for instance radio labelled prostaglandin, or did you only isolate the metabolites of endogenous prostaglandins?

Lerner. As we described above, incubations were performed with tritiated materials and then isolated and counted the radioactivity of the reaction products.

Crastes de Paulet. Yes, and you isolate 15 keto derivatives in prostaglandin metabolism?

Lerner. Yes.

Crastes de Paulet. Now, I have another question: have you looked at the activity of the isoquinoline derivatives on 15 hydroxyprostaglandin dehydrogenase of placenta, lung and kidney? Because kidney metabolism of prostaglandins is very complicated.

Lerner. We have studied but as yet not published the results of investigations on the effect of the inhibitors on human placental prostaglandin dehydrogenase in homogenates.

Crastes de Paulet. So your interpretation is that the effect on prostaglandin metabolism can come from a *direct* inhibition on these enzymes on PGDH.

Lerner. We believe that the effect on prostaglandin metabolism is at least in part, due to inhibition of the prostaglandin dehydrogenase.

Crastes de Paulet. And not on the biosynthesis of the enzyme?

Lerner. We cannot exclude the possibility that the compounds act on the biosynthesis of the enzyme but we have not performed studies to elucidate such information.