INFLUENCE OF α -MELANOCYTE-STIMULATING HORMONE (α -MSH), GROWTH HORMONE (GH) AND FETAL BRAIN EXTRACTS ON INTRA- UTERINE GROWTH OF FETUS AND PLACENTA IN THE RAT

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Summary

In order to determine what factors of the fetal hypothalamo-hypophyseal system can stimulate fetal body and placental growth, various compounds were tested. Fetal brain extracts, α -melanocyte-stimulating hormone (α -MSH) and growth hormone (GH) were injected directly into 19-day-old intact or brain-aspirated rat fetuses. Fetal and placental weight were determined on day 21. In addition, the effects of the operation and injection procedure itself on fetal and placental weight were established.

Increased placental weight was produced in brain-aspirated fetuses by α -MSH or GH, and in intact fetuses by GH and fetal brain extracts. Increased fetal weight was produced by α -MSH in brain-aspirated fetuses. These results suggest a possible role for GH and α -MSH in placental growth, and for α -MSH in fetal growth.

EXPERIMENTAL destruction of the fetal hypophysis (Liggins and Kennedy, 1968), the fetal hypothalamus (Fujita *et al.*, 1970) or the entire fetal brain (Swaab and Honnebier, 1973) in various animals, as well as observations on human anencephalic infants (Honnebier and Swaab, 1973) suggested to us that the fetal hypothalamo-hypophyseal system stimulates fetal and placental growth during intrauterine life (for review see Swaab and Honnebier, 1974).

In the present study we investigated the effect of fetal brain extracts, growth hormone (GH) and α -melanocyte-stimulating hormone (α -MSH) on fetal and placental growth. In addition, the effect of the operation and injection procedure on fetal and placental weight was determined.

In order to overcome any weight increase by oedema (Jost, 1954), dry weight measurements were included in most of the experiments.

MATERIALS AND METHODS

General procedure

Virgin female Wistar rats (T.N.O., Zeist, Netherlands), weighing approximately 180 g. each were housed at 25 °C. and exposed to 12 hours of light daily (from 0700 to 1900 hours). The animals received tap water and rat pellets (Hope Farms) *ad libitum*. They were mated overnight with male rats of the same strain. The day on which spermatozoa were found in the vaginal smear was taken as day 0 of pregnancy.

Operative procedures and fetal injections

The animals were divided at random into the various groups and were operated or sham operated at day 19 of pregnancy between 0900 and 1100 hours under general anaesthesia with Hypnorm (Philips-Duphar). This is a neuroleptic analgesic containing fluanisone and the analgesic phentanyl citrate.

The fetuses were left intact or brain-aspirated using a 1.1×38 mm. needle (Swaab and Honnebie, 1973) after which they received immediately by subcutaneous injections into the back through a 0.5×16 mm. needle the various test substances, dissolved in a volume of 50 μ l. (Heggstad and Wells, 1965; Noumura, 1959) of a sterile 0.9 per cent saline solution. The control fetuses received 50 μ l. of 0.9 per cent saline only.

Effect of the operation procedure

The effect of the operation and injection procedure on intrauterine growth was determined by comparing the fetal and placental weights of 5 litters of untouched mothers with those of 5 litters that were sham operated and subsequently injected with 50 μ l. 0.9 per cent saline. Sham operations were performed by only touching the fetal skulls with the same needle that was used for brain aspiration.

Wet and dry weight measurements

Live fetuses and their placentae were weighed with a sensitivity of 0.01 g. immediately after removal from the uterus on day 21. The fetal membranes and umbilical cord were trimmed before weighing. In order to correct the fetal weights for differences in cranial filling between the groups, each fetus was only weighed after emptying the cranial cavity by aspiration (Swaab and Honnebie, 1973). Subsequently, fetuses and placentae were kept in a hot-air incubator at 80 °C. for 4 days, in order to obtain dehydrated tissues. Fetal dry weight was determined with a sensitivity of 0.01 g. and placental dry weight with a sensitivity of 0.001 g. In order to determine when the fetuses or placentae would attain their dry weights, measurements were made on the litters of three intact untouched control mothers, three 50 μ l. physiological saline-injected and three 150 μ g. of α -MSH-injected intact litters.

Preparation of fetal brain extracts

Pregnant rats were killed by decapitation on day 20 of pregnancy and 431 fetuses were removed from the uterus and decapitated. The entire fetal brain was dissected out from all animals in the litter within five minutes, immediately frozen in liquid nitrogen and stored at -90 °C. The collected fetal brains were homogenized and extracted in HCl (Pecile *et al.*, 1969; Taleisnik and Tomatis, 1967), using an Ultra-Turrax homogenizer. This extraction was performed at 4 °C. for $2\frac{1}{2}$ hours in 100 μ l. of 0.1 N HCl per fetal brain. The extract was centrifuged at 10,000 revolutions per minute for 15 minutes and the supernatant was freeze-dried overnight. Before injection, the powder was dissolved in physiological saline and neutralized with NaOH at a pH of 7.4 in such volume that 50 μ l. was equivalent to the extract of 4 fetal brains.

After removal of the fetal brains for the extraction procedure, some fetal skulls were fixed in 4 per cent formalin. Longitudinal cryostat sections 15 μ in thickness were made and showed that the fetal hypophysis remained attached to the skull.

Statistics

Differences in fetal and placental weight between controls and the various test groups were tested by the Student "t" test (De Jonge, 1963) and $p < 0.05$ was considered to be statistically significant.

RESULTS

Measurement of dry weight (Tables I and II)

The decreasing fetal and placental weight, as determined during 5 days of drying at 80 °C. is given in Tables I and II. Only small weight changes were observed after the second day of drying.

Influence of operation and injection procedure on fetal and placental weight (Tables III and IV; Experiment I)

As a result of the operation and injection procedure, the fetal wet weight was 9.6 per cent less and the placental wet weight 8.6 per cent less than in intact untouched fetuses. Smaller

TABLE I
Fetal weight during drying at 80 °C.

Group	Wet fetal weight	Fetal weight after drying at 80 °C. for							
		1 day		2 days		4 days		5 days	
		Mean (and SEM) (mg.)	Mean (and SEM) (mg.)	Per cent of wet weight on day 0	Mean (and SEM) (mg.)	Per cent of wet weight on day 0	Mean (and SEM) (mg.)	Per cent of wet weight on day 0	Mean (and SEM) (mg.)
Untouched controls N=34	4,490 (45)	670 (7.2)	15.0	630 (5.6)	14.1	620 (5.1)	13.8	610 (5.1)	13.6
Fetuses injected with physiological saline N=35	4,240 (72)	630 (12.9)	15.0	590 (10.0)	13.9	580 (10.4)	13.7	570 (9.2)	13.4
Fetuses injected with 150 µg. of α-MSH N=34	4,300 (38)	610 (6.3)	14.3	580 (5.8)	13.6	570 (6.1)	13.3	570 (6.1)	13.2

TABLE II
Placental weight during drying

Group	Wet placental weight	Placental weight after drying at 80 °C. for							
		1 day		2 days		4 days		5 days	
		Mean (and SEM) (mg.)	Mean (and SEM) (mg.)	Per cent of wet weight on day 0	Mean (and SEM) (mg.)	Per cent of wet weight on day 0	Mean (and SEM) (mg.)	Per cent of wet weight on day 0	Mean (and SEM) (mg.)
Untouched control N=34	570 (12.1)	83 (2.1)	14.5	83 (2.0)	14.5	82 (2.0)	14.3	82 (2.0)	14.3
Fetuses injected with physiological saline N=35	500 (12.4)	77 (1.7)	15.4	77 (1.6)	15.3	76 (1.6)	15.1	76 (1.6)	15.0
Fetuses injected with 150 µg. of α-MSH N=34	530 (14.3)	79 (1.7)	15.0	78 (1.7)	14.8	77 (1.7)	14.7	77 (1.7)	14.6

TABLE III

Changes in fetal wet and dry weight, as a result of the operation and injection procedure (experiment I), after injection of fetal brain extracts (Experiments II and III) or after injection of GH and α -MSH (Experiments IV to XI)

Experiment	Treatment	Experimental group						Control group						Result trend in experimental group	
		Mothers	Living fetuses	Deceased fetuses (per cent)	Wet weight (g.) Mean (and SEM)	Dry weight (mg.) Mean (and SEM)		Mothers	Living fetuses	Deceased fetuses (per cent)	Wet weight (g.) Mean (and SEM)	Dry weight (mg.) Mean (and SEM)		Wet weight	Dry weight
I	Sham operation	5	53	7.5	4.13 (0.071)	570 (11.2)	****	5	55	0	4.57 (0.040)	620 (5.1)		↓	↓
II	Injection of fetal brain extracts into intact fetuses	7	47	33.8	3.45 (0.055)	460 (8.1)	****	6	67	0	3.78 (0.045)	500 (7.2)		↓	↓
III	Injection of fetal brain extracts into brain aspirated fetuses	4	36	16.2	3.01 (0.063)	390 (9.4)	****	7	68	1.4	3.41 (0.043)	470 (6.4)		↓	↓
IV	Injection of 150 μ g. of GH into intact fetuses (NIAMD-RAT-G-B-1)	5	41	2.4	4.02 (0.082)	n.d.	**	5	50	2.0	4.13 (0.055)	n.d.		↓	n.d.
V	Injection of 150 μ g. of GH into brain aspirated fetuses (NIAMD-RAT-G-B-1)	6	45	22.4	2.65 (0.063)	n.d.	**	5	51	8.9	2.87 (0.053)	n.d.		↓	n.d.
VI	Injection of 15 μ g. of GH into brain aspirated fetuses (NIH-GH-B17)	5	57	1.7	3.40 (0.034)	470 (5.2)	*	5	51	1.9	3.54 (0.038)	500 (5.9)		↓	↓
VII	Injection of 150 μ g. of GH into brain aspirated fetuses (NIH-GH-B17)	5	32	23.3	3.62 (0.070)	490 (9.8)		5	51	1.9	3.54 (0.038)	500 (5.9)		—	—
VIII	Injection of 150 μ g. of α -MSH into intact fetuses	5	57	7.0	4.20 (0.030)	560 (4.2)		5	53	1.7	4.13 (0.071)	567 (11.1)		—	—
IX	Injection of 150 μ g. of α -MSH into brain aspirated fetuses	9	84	10.7	3.38 (0.043)	n.d.	****	8	75	12.5	2.92 (0.039)	n.d.		↑	n.d.
X	Injection of 15 μ g. of α -MSH into brain aspirated fetuses	4	38	11.6	3.41 (0.053)	480 (7.5)	*	5	53	10.2	3.22 (0.063)	450 (8.9)		↑	↑
XI	Injection of 150 μ g. of α -MSH into brain aspirated fetuses	4	47	2.1	3.52 (0.053)	470 (8.7)	****	5	53	10.2	3.22 (0.063)	450 (8.9)		↑	—

n.d. = not determined.

Significances of difference between test and control group as determined by means of Student's "t" test are:

* $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$; **** $p < 0.005$; ***** $p < 0.001$.

TABLE IV
Changes in placental wet and dry weight as a result of the operation and injection procedure (experiment I), after injection of fetal brain extracts (Experiments II and III) or after injection of GH and α -MSH (Experiments IV to XI)

Experiment	Treatment	Experimental group					Control group					Result trend in experimental group	
		Mothers	Living fetuses	Deceased fetuses (per cent)	Wet weight (g.) Mean (and SEM)	Dry weight (mg.) Mean (and SEM)	Mothers	Living fetuses	Deceased fetuses (per cent)	Wet weight (g.) Mean (and SEM)	Dry weight (mg.) Mean (and SEM)	Wet weight	Dry weight
I	Sham operation	5	53	7.5	0.53 (0.011)	79 (1.7)	5	55	0	0.58 (0.009)	83 (1.5)	↓	—
II	Injection of fetal brain extracts into intact fetuses	7	47	33.8	0.53 (0.010)	79 (1.5)	6	67	0	0.51 (0.008)	75 (1.1)	↑	↑
III	Injection of fetal brain extracts into brain aspirated fetuses	4	36	16.2	0.50 (0.013)	68 (2.1)	7	68	1.4	0.53 (0.008)	76 (1.4)	—	↓
IV	Injection of 150 μ g. GH into intact fetuses (NIAMD-RAT-G-B-1)	5	41	2.4	0.55 (0.012)	n.d.	5	50	2.0	0.51 (0.009)	n.d.	↑	n.d.
V	Injection of 150 μ g. of GH into brain aspirated fetuses (NIAMD-RAT-G-B-1)	6	45	22.4	0.54 (0.014)	n.d.	5	51	8.9	0.49 (0.011)	n.d.	↑	n.d.
VI	Injection of 15 μ g. of GH into brain aspirated fetuses (NIH-GH-B17)	5	57	1.7	0.53 (0.009)	80 (1.4)	5	51	1.9	0.53 (0.008)	78 (1.5)	—	—
VII	Injection of 150 μ g. of GH into brain aspirated fetuses (NIH-GH-B17)	5	32	23.3	0.55 (0.017)	81 (2.7)	5	51	1.9	0.53 (0.008)	78 (1.5)	—	—
VIII	Injection of 150 μ g. of α -MSH into intact fetuses	5	57	7.0	0.52 (0.009)	77 (1.2)	5	53	1.7	0.53 (0.011)	79 (1.7)	—	—
IX	Injection of 150 μ g. of α -MSH into brain aspirated fetuses	9	84	10.7	0.51 (0.007)	n.d.	8	75	12.5	0.45 (0.006)	n.d.	↑	n.d.
X	Injection of 15 μ g. of α -MSH into brain aspirated fetuses	4	38	11.6	0.54 (0.008)	79 (1.2)	5	53	10.2	0.51 (0.007)	77 (1.1)	↑	—
XI	Injection of 150 μ g. of α -MSH into brain aspirated fetuses	4	47	2.1	0.53 (0.008)	75 (1.1)	5	53	10.2	0.51 (0.007)	77 (1.1)	—	—

n.d. = not determined.

Significances of difference between test and control groups as determined by Student's "t" test are:

* $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$; **** $p < 0.005$; ***** $p < 0.001$.

decreases (7.9 per cent and 4.8 per cent respectively) were found in fetal and placental dry weights. The decrease in placental dry weight just failed to reach statistical significance ($0.05 < p < 0.10$).

Influence of fetal brain extracts on intrauterine growth (Tables III and IV; Experiments II and III)

The fetal brain extracts diminished fetal wet and dry weight in both intact and brain-aspirated fetuses. A significant increase was observed, however, in placental wet and dry weights after administration of fetal brain extracts to intact fetuses. This increased placental weight was not found in brain-aspirated fetuses. Placental wet weight even decreased in the latter group.

Influence of growth hormone (GH) on fetal and placental weight (Tables III and IV; Experiments IV and VII)

Fetal body weight was reduced in intact as well as in brain-aspirated fetuses by rat GH administration in three experiments (IV, V and VI) and was unaffected in one group (VII).

Rat GH induced a clear-cut increase in placental wet weight in intact as well as in brain-aspirated fetuses (Experiments IV and V). Bovine GH did not produce any significant placental wet or dry weight increases (Experiments VI and VII).

Influence of α -MSH on fetal and placental weight (Tables III and IV; Experiments VIII to XI)

In intact fetuses 150 μ g. of α -MSH had no effect on fetal or placental weight. In brain-aspirated fetuses, however, fetal wet weight (Experiments IX to XI) and dry weight (Experiment X) were significantly increased in the α -MSH-treated groups. The increased placental wet weight and fetal dry weight of experiment XI were at the border of statistical significance ($0.05 < p < 0.10$). The trend towards increased placental wet weight (Experiments X and XI) was, however, not reflected in increased dry weight.

DISCUSSION

Many environmental and maternal factors are known to influence intrauterine growth (Knobil

and Caton, 1953; Kloosterman, 1966). In addition, the fetal brain itself seems to stimulate fetal body and placental growth (Honnebier and Swaab, 1973; Swaab and Honnebier, 1973 and 1974). Lesion studies in sheep (Liggins and Kennedy, 1968) and rats (Fujita *et al.*, 1970) suggest that the fetal brain exerts this influence by the hypothalamo-hypophyseal system. Observations on human anencephalic infants have confirmed the importance of the fetal brain for intrauterine growth (Honnebier and Swaab, 1973). Birthweight in this group appeared to be less than that of children with other types of serious congenital anomalies (Swaab and Honnebier, 1974). Moreover, a study of twin pregnancies in which one fetus was anencephalic showed that the decreased birthweight in anencephalic infants is not caused by an alteration in maternal environment (Honnebier and Swaab, 1973). These experimental and clinical data, and the role of the hypothalamo-hypophyseal system in postnatal growth (Van der Werff ten Bosch, 1966) led to our interest in studying the effect of certain substances upon fetal and placental growth.

Fetal oedema has been reported (Jost, 1954) after intrauterine operations and other experiments during pregnancy. Dry weights were therefore determined in some experiments. Our operation and injection procedure did not appear to cause oedema in our experiments (Tables III and IV). On the contrary (but in agreement with Jost and Picon, 1957) fetal weight and dry weights were clearly lower in the operated group, while placental weight appeared to be lower in sham operated animals. Indeed if a litter was in poor condition, body and placental weights were not only lower than normal but also showed no evidence of excess fluid retention. The mechanisms by which operation and injection procedures affect intrauterine growth are not known. Changes in uterine blood flow (Bruce, 1972) or food (Jost and Picon, 1957) and maternal water intake (Boer *et al.*, 1974) may play a role.

In the present study all fetal skulls were aspirated prior to weighing in order to allow comparisons of body weight between intact and brain-aspirated fetuses.

The fact that no increase in fetal growth was obtained following administration of growth hormone (GH) indicates that fetal growth is

most probably not regulated by the same mechanisms as infant growth (Van der Werff ten Bosch, 1966). The results obtained by Heggestad and Wells (1965) might be explained by contamination of their GH preparation with α -MSH particularly as the stimulant effect was only seen in decapitated fetuses. That GH does not stimulate fetal growth is in agreement with clinical observations. Ateleotic dwarfs have normal birthweights (Rimoin *et al.*, 1966), while even a negative relation exists between fetal growth hormone levels and birthweight (Cramer *et al.*, 1971).

GH produces a clearcut increase in placental wet weight in intact as well as in brain-aspirated fetuses. Relevant to the effect of GH is the trend towards heavier placentae observed after GH administration to pregnant rats (Croskerry *et al.*, 1973). This observation, together with the finding that maternal hypophysectomy induces a decreased placental weight (Knobil and Caton, 1953) suggest that not only fetal but also maternal GH will influence placental weight. GH releasing factor (GH-RF) activity was found in stalk median eminence extracts of neonatal rats in high concentration (Pecile *et al.*, 1969). The increased placental growth observed in the present study after injection of such extracts in intact, but not in brain-aspirated fetuses who have no pituitary (Swaab and Honnebier, 1973) can also be explained by the effect of GH-RF on the pituitary of the intact animal. The finding that in human anencephalic infants both low placental weight (Honnebier and Swaab, 1973) and low GH levels (Grumbach and Kaplan, 1973) were observed suggests that GH may stimulate placental growth in humans as well.

A striking increase in fetal body weight was obtained by α -MSH. This effect, never previously reported, was only found in brain-aspirated fetuses. The 6 to 16 per cent wet weight increase induced by a single injection of α -MSH was not as large as the 25 to 33 per cent weight decrease caused by brain aspiration (Swaab and Honnebier, 1973). The slight increase in placental wet weight after α -MSH administration was also found only in brain-aspirated fetuses. This increase was, however, not reflected in increased dry weight. Since the placentae were not pale or oedematous and the fetuses were heavier after α -MSH treatment, the increased placental weight

might have been caused by an increased blood content. Significant in this respect is the effect of α -MSH on the cardio-vascular system (Aldinger *et al.*, 1973). The exact mechanism by which α -MSH exerts its effect remains to be elucidated.

Among the different forms of MSH in the pituitary of the rat, the α -form seems to be more important than the β -form (Baker, 1973; Shapiro *et al.*, 1972) although other forms of MSH will probably also exist (Shapiro *et al.*, 1972; Thody, 1969). No clear physiological function has been established up till now for the intermediate lobe hormone in mammals. Various extra-pigmentary effects of MSH are, however, described in the literature, such as effects on the thyroid (Akgün *et al.*, 1969; Bowers *et al.*, 1964; Cehovic, 1966), adipokinetic effects (Rudman *et al.*, 1963), effects on behaviour (Dempsey *et al.*, 1972), on the nervous system (Krivoy *et al.*, 1963), on heart rate (Aldinger *et al.*, 1973) and on the eyes (for review see Novales, 1967). Our data suggests a new role for MSH, namely the stimulation of fetal growth. This effect will probably not be based upon the adipokinetic effect of MSH, since ACTH and TSH both of which also possess high adipokinetic activity (Rudman *et al.*, 1963) had no stimulatory effect on fetal growth (Swaab and Honnebier, 1974). The idea of a possible role of MSH in the physiology of fetal development is supported by the early presence of MSH in human (Kastin *et al.*, 1968; Levina, 1968) and in mouse pituitaries (Enemar, 1963) and by the finding of a direct relationship between body weight and the amount of MSH in the pituitary in the postnatal period (Tilders, 1973).

If MSH were indeed the main substance via which brain stimulates fetal growth in the rat, one would expect that around 19 to 21 days of fetal life in the rat, MSH release would mainly be controlled by a releasing factor. This would explain the augmentation of fetal growth produced by the fetal hypophysis (Liggins and Kennedy, 1968) and the hypothalamus in sheep (Fujita *et al.*, 1970) and the fact that the growth inhibition seen in encephalotomized rats which still have a pituitary is at least as strong as in decapitated rats (Dupouy and Jost, 1970).

The placental growth that occurred after administration of fetal brain extracts to intact fetuses may be explained by postulating that the

extracts contained GH-RF. The absence of fetal growth increase after administration of fetal brain extract to intact fetuses suggested that MSH production can only be augmented in fetuses whose pituitary is deprived of releasing factor. The hypothesis that our fetal brain extracts do indeed contain MSH-RF, and can stimulate fetal growth needs thus to be confirmed in encephalotomized fetuses, and by *in vitro* techniques for MSH-RF determination.

MSH has been found in the fetal hypophysis (Kastin *et al.*, 1968; Levina, 1968; Enemar, 1963) and the maternal hypophysis (Karkun and Sen, 1965; Lee and Lee, 1969), while the placenta (Dahlberg, 1961) and the brain (Rudman *et al.*, 1973) seem to contain MSH-like compounds. The paradox that fetal growth is inhibited in anencephalics while being normal in human fetuses with hypophysial aplasia, could mean that MSH can come from non-pituitary sources under the influence of an intact brain.

In conclusion, we believe that it has been possible to show in the rat that GH stimulates placental growth while MSH mainly stimulates fetal growth. The results of GH and MSH determinations on fetal or infant blood in normal and pathological human pregnancies should give some evidence about the role of GH and MSH in development of the human fetus and placenta.

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