Exclusion of the fetal brain as the main source of rat and human amniotic fluid oxytocin

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Summary. At term relatively high oxytocin concentrations are found in maternal plasma and in rat and human amniotic fluid. To determine the contribution of the fetal brain to these oxytocin levels, the peptide was measured in maternal rat plasma and amniotic fluid 2 days after intra-uterine removal of the fetal brains, and in the amniotic fluid of 16 human anencephalics. After removal of the fetal rat brains and in human anencephalic pregnancies normal maternal plasma concentrations and amniotic fluid oxytocin contents were found. Consequently, both maternal plasma oxytocin and amniotic fluid oxytocin are not determined to any substantial degree by the fetal brain.

In late pregnancy relatively high concentrations of oxytocin are present in maternal blood and amniotic fluid, whereas high arginine-vasopressin (AVP) concentrations are found in umbilical cord blood. The contribution of the fetal brain to the neurohypophyseal hormone levels in these compartments was studied by removing the fetal brain in the rat, and by the effect of anencephaly in 16 human pregnancies.

Methods

Experimental procedures

Wistar rats were mated overnight. The day on which spermatozoa were found in the vaginal smear was taken as day zero of pregnancy [for details see Swaab & Honnebier (1973)]. The operations as well as the collection of blood and amniotic fluid were performed between 09.00 and 11.00 hours. Forty-five animals were killed by decapitation and maternal blood was collected from the body stump in pre-chilled plastic tubes containing 100 units/tube Na-heparin. Blood was immediately centrifuged at 1600 g for 15 min at 4°C, after which plasma was separated and kept

frozen at -15°C until assayed. Amniotic fluid was collected immediately after decapitation of the pregnant rat by aspirating each amniotic compartment separately using a gauge 19 needle and a 1 ml plastic syringe. The amniotic fluid was pooled per litter and frozen immediately after collection in pre-chilled plastic tubes and kept at -15°C until assayed. The mean storage time of the rat samples was 11 days (range 0–32 days).

The fetal brains were removed on day 19 of pregnancy by aspiration. For sham operation the same procedure was followed, the fetal skull only being touched by the needle [for details on this procedure see Swaab & Honnebier (1973)]. All sham-operated fetuses, and all but one of the 117 brain-aspirated fetuses, survived the operation.

Human anencephalics

Amniotic fluid has been collected during past years for group I anencephalics and stored immediately at -15°C. Mixed arterio-venous umbilical blood and maternal venous antecubital blood were taken (after informed consent of the mother) immediately after delivery and collected in pre-chilled heparinized tubes (containing 125 units lithium-heparin). Plasma was prepared, as above, and kept at -15°C until assayed. The

Table 1. Human anencephalic data (group I)

Patient No.	Sex	Gestation length (weeks)	Birth-weight (g)	Hydram-nios	Ind. labour	Oxytocin (pg/ml)			AVP (pg/ml)		
						MAT	UMB	AMN	MAT	UMB	AMN
26-1-47	M	15 3/7	290	—	OXT	41	<86	ND	<2	<24	ND
370211**	M	26	555	—	—	22	47	19	<5	<5	<5
566	F	35	1060	+	OXT	69	ND	<2	<3	ND	<9
Rd-2	F	35 4/7	1710	+	OXT	46	ND	<0.25	ND	ND	<0.25
1-1415*	F	39	2110	—	—	44	ND	31	10	ND	<2
12-6-59	F	39 4/7	1330	+	—	<25	51	33	<9.7	2	<2
Rd-1	M	41 1/7	2780	—	—	ND	0	63	ND	ND	ND
7-10-55	M	44	3180		PGE ₂	42	22	71	<2	<2	<2

+, Present; —, absent; Ind, induction of labour; OXT, oxytocin; AVP, vasopressin; PGE₂, prostaglandin E₂; MAT, maternal plasma level; UMB, umbilical cord plasma level; AMN, amniotic fluid level; ND, not determined (no sample available).

Samples collected in patients who were not in labour: *Amniocentesis; **elective caesarean.

samples of group I were stored for a period of 6.5 weeks, range 1–10 weeks. Group II consisted of eight amniotic fluid samples collected by amniocentesis before labour between 1974 and 1982 and kept at -15°C (mean storage time 217 weeks, range 18–415 weeks), together with 10 controls matched for storage time and gestation length. This material has been kindly donated by Prof. H. Huisjes (Groningen). Clinical data on the anencephalic material are presented in Tables 1 and 2.

Radioimmunoassay

AVP and oxytocin were measured in the same sample, in duplicate, after Vycor extraction by highly specific radioimmunoassays as described previously (Dogterom *et al.* 1980). The limit of detection was 1 pg for oxytocin and 0.5 pg for AVP per 2 ml serum or amniotic fluid. The inter-assay coefficient of variation for AVP and oxytocin (after extraction of the samples) was 13.9% and 8.6% respectively, while the intra-assay coefficient of variation (for unextracted AVP and oxytocin samples) was 15.7% and 10.2% respectively. These data were all obtained with 16–32 pg samples.

The Mann–Whitney *U*-test (corrected for ties) was used to calculate differences and Spearman rank for correlations, considering $\alpha < 0.05$ (two-tailed) as being significant.

Table 2. Amniotic fluid levels of oxytocin and vasopressin (AVP) in human anencephalics and controls (group II)

Patient No.	Date of amnio- centesis	Gestation length (weeks)	Amniotic fluid	
			Oxytocin (pg/ml)	AVP (pg/ml)
Anencephalics				
1444	31-1-80	26	22	<6
1483	28-3-80	27	19	<7
1944	20-1-81	32	18	<4
118	21-11-74	34	26	<7
1653	20-10-80	35	15	<5
111	8-11-74	36	17	<5
76	27-6-74	42	17	<7
72	1-6-74	>42*	<10*	<5
Mean (SEM)		34(2)	18(2)	
Control group				
1474	12-3-80	32	26	<4
1905	2-12-81	32	27	<4
1680	29-12-80	33	20	<4
1931	11-1-82	34	<10*	<5
169	19-3-75	34	19	<4
1634	21-10-80	34.5	14	<4
105	10-10-74	35	16	<4
116	15-11-74	35	19	<4
92	6-9-74	35.5	11	<4
1624	14-10-80	37	16	<4
Mean (SEM)		34(1)	18(2)	

*For calculation of the mean the detection levels were taken as values.

Results

The amount of amniotic fluid in the non-operated control animals decreased steadily from 3.5 ml per litter at day 19 to 1.6 ml at day 21 (Fig. 1).

During the (sham-) operation all amniotic fluid leaked out of the uterus through the needle holes, but two days later the holes could not be seen and the uterine and fetal compartments were fully closed, again containing some amniotic fluid. In addition, the holes in the fetal heads of the brain-aspirated animals were completely closed. From sham-operated fetuses only 100 μ l amniotic fluid/litter could be obtained on day 21, whereas from brain-aspirated litters about six times more fluid was obtained ($P = 0.0012$).

Maternal plasma oxytocin on day 19–21 of gestation in the non-operated control animals (Fig. 2) revealed no significant difference between day 19–20 ($P = 0.63$) or 19–21 ($P = 0.16$). However, the rise between days 20 and 21 was

significant ($P = 0.024$). The plasma concentrations of the (sham-) operated and brain-aspirated animals were not significantly different from the oxytocin plasma concentrations of the non-operated control animals at day 21 ($P = 0.21$ and $P = 0.19$ respectively). The maternal plasma concentrations of AVP were so often below the level of detection that no conclusions could be drawn.

The amniotic fluid oxytocin concentrations at day 19–21 of gestation in the non-operated control animals were about twice as high as the maternal blood concentrations (Fig. 3), while the AVP levels were spread around 25 pg/ml. No significant difference was found in the oxytocin concentration from day 19 to 21 (day 19–20, $P = 0.49$; day 20–21, $P = 0.63$). In the sham-operated animals roughly eight times higher levels of oxytocin ($P = 0.005$) and AVP ($P = 0.007$) were found in the amniotic fluid as compared with

Fig. 1. The volume of amniotic fluid per rat litter in (a) the unoperated control animals at days 19, 20 and 21 of gestation and (b) at day 21 after sham operation (Sham) or fetal brain aspiration (Asp) performed at day 19 of gestation. The bars indicate the median value, the dots represent the individual observations.

Fig. 2. Maternal plasma oxytocin (▨) and AVP (□) concentrations in (a) the unoperated control animals at days 19, 20, and 21 of gestation and (b) in the sham-operated (Sham) and fetal-brain aspirated (Asp) animals at day 21 of gestation. The bars indicate the median value, the dots represent individual observations, with ▽ values below detection level.

the unoperated day-21 control group. Brain aspiration caused a decrease of oxytocin concentrations to 30% of the sham-operated levels ($P = 0.01$), but still remained 2.5 times higher than in unoperated day-21 rats ($P = 0.003$). The AVP amniotic fluid levels were so frequently below detection level that they did not allow any conclusions.

In view of the large changes in amniotic fluid volume observed during the last days of pregnancy and after the operative procedures, the absolute amounts of these peptides per litter were calculated (Fig. 4). This revealed in the non-operated animals a non-significant drop in total amniotic oxytocin from 270 pg/litter on day 19 to 160 pg/litter on day 20 ($P = 0.35$) and for AVP a significant decrease from 80 pg/litter to 20

pg/litter ($P = 0.001$). Neither the amount of oxytocin ($P = 0.63$) nor that of AVP ($P = 0.29$) changed between day 20 and 21.

No differences were found for the total amount of oxytocin in amniotic fluid between sham-operated and non-operated control animals ($P = 0.27$, and between sham-operated and brain-aspirated litters ($P = 0.25$).

When the data were calculated as the total amount of peptide in amniotic fluid per fetus, in order to correct for the small differences in litter size (the average litter size ranged from 10.1 to 12.4, the standard error of the mean from 0.3 to 1.1), exactly the same results as those of Fig. 4 were obtained.

Control values $\pm$ SEM were obtained from 31 patients at 33–41 weeks gestation. The mean

Fig. 3. Amniotic fluid levels of oxytocin (▨) and AVP (□) in (a) the unoperated control animals at days 19, 20 and 21 of gestation and (b) in sham-operated (Sham) and fetal-brain aspirated (Asp) animals at day 21 of gestation. The bars indicate the median value, the dots represent individual observations, ▽ indicates values below, and ▲ values above detection level.

amniotic fluid oxytocin concentration in the group I anencephalics (see Table 1) (31 ± 10 pg/ml) was within the control range (63 ± 35 pg/ml). AVP was undetectable in all anencephalics (control range 2 ± 1 pg/ml). Only once was a very low amount, near detection level, of AVP found in the umbilical cord blood (normal level 191 ± 47 pg/ml), while oxytocin was found in three out of the five determinations to be within the control range of 33 ± 6 pg/ml. Maternal AVP and oxytocin levels were within the control range (4 ± 1 pg/ml and 70 ± 10 pg/ml, respectively). In group II anencephalics oxytocin was demonstrable in all amniotic fluid samples, except one (Table 2), while AVP was always below the limit of detection. The amniotic oxytocin levels in the anencephalics (18 ± 2 pg/ml), were similar to those in the controls (18 ± 2 pg/ml). In addition,

these values agree very well with the control values obtained by amniocentesis performed in patients before labour (20 ± 4 pg/ml).

Discussion

The relatively high maternal plasma oxytocin concentrations in the rat and man in late gestation (Swaab & Boer 1979) seem not to be determined in any substantial way by the fetal brain, since after removal of the fetal rat brain and in human anencephalics, maternal plasma oxytocin levels were within the normal range. The disappearance of human fetal oxytocin as calculated from the arterio-venous difference in the umbilical cord blood (Dawood *et al.* 1978) thus seems to make either an insignificant contribution to the maternal levels, or is largely due to

Fig. 4. The total amount of oxytocin (▨) and AVP (□) in amniotic fluid per litter; (a) during the last days of gestation and (b) in sham-operated (Sham) and fetal brain aspirated (Asp) animals at day 21 of pregnancy. The bars indicate the median value, the dots represent individual observations, and ▽ indicates values below detection level.

placental binding and/or degradation. The presence of oxytocin in amniotic fluid of human anencephalics shows that this peptide comes either from the maternal compartment or from a fetal source other than the brain (see below). The very high oxytocin levels in the amniotic fluid of sham-operated animals can be explained by the total disappearance of amniotic fluid during the operation and the small amount of amniotic fluid produced thereafter, in which the peptide is concentrated. When the total amount of oxytocin present in brain-aspirated rat amniotic fluid at term was calculated, it was not different from that of sham-operated controls nor from that of the unoperated animals at term.

Four alternative sources have been supposed to contribute to the normal amniotic fluid levels of

oxytocin and AVP, fetal urine being one of them, but since the human amniotic fluid oxytocin concentration was twice that of fetal urine, the latter was not considered to be the sole source (Dawood *et al.* 1979b). On the other hand, higher oxytocin levels were found in the fetal urine than in amniotic fluid (Seppala *et al.* 1972), which lays more emphasis on fetal urine being the possible source of amniotic oxytocin. The present study indicates that if the fetal urine determines amniotic oxytocin to any substantial degree, the main source will not be the fetal brain. Although bioassays revealed the presence of oxytocin in the human placenta up to the 15th week of gestation (Levina 1968) the placenta at term is usually excluded as a site of production for oxytocin, because of the higher artery than vein levels of

oxytocin in the umbilical blood (Dawood *et al.* 1978) and the absence of oxytocin and AVP in the umbilical cord plasma of one anencephalic (Chard *et al.* 1971). The latter point needs to be reinvestigated because of the presence of oxytocin in the umbilical cord blood in three out of five anencephalics (Table 1). The literature disagrees about whether maternal oxytocin might reach the fetal compartment, but perfusion of oxytocin in the ewe did not increase the fetal blood levels and vice versa (Glatz *et al.* 1980). Finally, amniotic fluid oxytocin might be produced by fetal organs other than the brain or by the fetal membranes. Whatever the source may be, our observation of the presence of oxytocin in the cord of three anencephalics without the concomitant high levels of AVP found in normal children argues in favour of the fetal circulation as the route for oxytocin passage to amniotic fluid. From the fetal circulation oxytocin may be efficiently transferred to the amniotic fluid as suggested by the rapid increase of oxytocin in the amniotic fluid after injection of oxytocin into an umbilical vessel of the baboon (Dawood *et al.* 1979a). As for AVP, the present experiments in the rat and man do not allow any final conclusion, because too many samples were below the detection limit.

The enhanced oxytocin levels in amniotic fluid that arise in the course of human labour (Seppala *et al.* 1972; Dawood *et al.* 1978) might also be derived either from the mother or from the fetus. If oxytocin in the amniotic fluid could act directly on the gravid uterus, the high levels found in late gestation in the rat and in man might well play a part in the initiation of labour. In this respect it might be significant that the decidua and amnion, and not the myometrium, were found to be the sites for the stimulating action of oxytocin on prostaglandin production (Fuchs *et al.* 1981). If oxytocin is indeed involved in the initiation of labour, fetal brain oxytocin does not seem to play a major role since the mean gestation length was not changed in the group of human anencephalics that did not have hydramnios (Honnebier & Swaab 1973) or in the rat after removal of the fetal brain (Swaab *et al.* 1977). On the other hand, in both human anencephalics and brain-aspirated rats, the course of labour was found to be protracted (Swaab *et al.* 1977), suggesting a role for fetal brain oxytocin on the course of normal labour. The results of the present study showing that oxytocin is present in the amniotic fluid in these conditions seems at the first sight to disprove such a role for oxytocin from this

compartment. But the contractile effects of oxytocin are mediated by receptors (Fuchs *et al.* 1981, 1982), which in the case of anencephaly might be present in fewer numbers as a result of the lower oestrogen/progesterone ratio (Alexandrova & Soloff 1980).

A most intriguing observation is the sixfold higher amount of amniotic fluid obtained from brain-aspirated fetuses compared with the sham-operated controls. Increased amounts of amniotic fluid are found in experimentally induced exencephaly and anencephaly in fetal rats and hamsters (see Ferm & Saxon 1971) and in 74% of human anencephalic pregnancies (Honnebier & Swaab 1973). The cause of this high incidence of hydramnios has never been adequately established. However, since in our rat experiments a larger amount of amniotic fluid was found, while no intercranial structure is present, this does not support the hypothesis that hydramnios in anencephaly is due to a leakage of cerebrospinal fluid from the choroid plexus-like structure on top of the anencephalic head (Taussig 1927). Our observations seem, in addition, to provide an experimental model to answer such questions as whether fetal neurohypophysial hormones may be involved in the regulation of the amount and composition of the amniotic fluid (Swaab *et al.* 1978).

Acknowledgments

We are grateful to The Van den Houten Fund and the Committee for the Furtherance of Veterinary and Comparative Pathological Research for their financial support, J. J. van Heerikhuizen for his technical assistance, Dr J. G. van Oyen for his statistical advice and W. Chen-Pelt and P. J. van Nieuwkoop for their secretarial help.

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Received 22 December 1982

Accepted 10 July 1983