Localisation of oxytocin, vasopressin and parts of precursors in the human neonatal adrenal

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Summary. Being a possible alternative source for the production of vasopressin (AVP) and oxytocin (OXT), a study was undertaken of the fetal adrenal. The concentrations of these peptides within the fetal adrenal turned out to be low, viz., approx. 1 pg/mg in the rat and within the pg/g range in the human. Immunocytochemistry was performed either on conventional autopsy material kept till 12 years in paraffin blocks, or on more recently obtained formalin or glutaraldehyde-paraformaldehyde fixed material. In both types of material staining was good. In order to localize AVP cells, anti-AVP, an antibody against its associated neurophysin (anti-NSN) or an antibody raised against the c-terminal glycopeptide part of the AVP precursor (anti-GP) was used. OXT cells were localized by means of anti-OXT or an auto-antibody of a multiple sclerosis patient (auto-MS) probably recognizing OXT-neurophysin. The antibodies were characterized on human and rat brain material. In the external zone of the definitive cortex, apart from parenchyma cells, anti-AVP, anti-NSN and anti-GP stained fibre-like structures running in the connective tissue septa and around parenchyma cells and the cytoplasm of these cells. Anti-OXT and auto-MS stained droplets in the cytoplasm of the fetal zone cells. Similar distinct staining patterns for AVP and OXT cells were obtained in human anencephalics. These observations show that the peptides are not derived from the fetal brain, but are rather produced in the fetal adrenal cortex. Future research will have to determine the physiological meaning of the presence of these peptides in the fetal adrenal, e.g., in their contribution to amniotic fluid peptides, their possible role in fetal stress, steroidogenesis etc. The presence of an alternative source of these peptides in the fetus makes it necessary, furthermore, to reconsider their possible functions in the process of labour.

Introduction

In late gestation, relatively high levels of oxytocin (OXT) are present in amniotic fluid (Swaab and Oosterbaan 1983). This peptide might be involved in the initiation and course of labour (Fuchs et al. 1981; Fuchs and Fuchs 1984). Recent observations have shown that the main source of am-

niotic fluid OXT is probably localized outside the fetal brain. After removal of the fetal rat brain and in human anencephalics amniotic fluid OXT is within the normal range (Swaab and Oosterbaan 1983). Since this peptide does not pass readily from the mother to the amniotic fluid in the rat (Oosterbaan et al. 1985a), and because it was found in umbilical cord plasma of some human anencephalics (Swaab and Oosterbaan 1983), it seems to be produced by fetal organs other than the brain. The source of amniotic vasopressin (AVP) was not made clear by our studies, because of detection problems due to its low levels.

The fetal adrenal was found by biochemical techniques to contain OXT and AVP (Nicholson et al. 1984). Since in fetal stress – a condition in which the adrenal is supposed to be activated (Serrón-Ferré and Jaffe 1981; Norman and Joubert 1982; Delprado and Baird 1984), – amniotic AVP levels increase (Stark et al. 1984; Oosterbaan et al. 1985b) the fetal adrenal was considered to be a possible alternative source for the amniotic fluid peptides. After determining, by means of radioimmunoassay, the concentrations of OXT and AVP in human and rat adrenals, as a first step in testing this hypothesis, normal human neonatal as well as anencephalic adrenals were studied immunocytochemically for the presence of OXT, AVP, and their related neurophysins and, for AVP its precursor or glycopeptide part (cf. Ivell and Richter 1984). In addition, some data in the fetal rat adrenal on AVP and OXT are reported.

Materials and methods

Human fetal adrenals. Routine autopsy material was used from seven control neonates (i.e. not having any gross brain pathology) and five anencephalics. The material was 4% formalin fixed, dehydrated and kept in paraffin blocks. In addition, three control adrenals and one from an anencephalic were fixed in 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M cacodylate buffer pH 7.4. For some clinical details see Table 1. Moreover, nine control adrenals were stored frozen after dissection until assayed.

Fetal rat material. Virgin female Wistar rats were obtained from CPB-TNO (Zeist, the Netherlands), and mated overnight with males of the same strain. The day on which spermatozoa were found in the vaginal smear was taken as day 0 of pregnancy. On the morning of day 21 of gestation, the pregnant mothers were decapitated, the fetuses were rapidly removed and 30 adrenals dissected and put into 0.1 M HCl for a single radioimmunoassay of OXT and AVP.

Table 1. Staining of oxytocin and vasopressin cells in the human neonatal adrenal

Patient Nr	Sex	Cause of death	Gestation [weeks] ^a	Weight [g]	Staining					
					anti- OXT [in FC]	auto- MS [in FC]	anti- AVP [in DC]	anti- NSN [in DC]	anti- GP [in DC]	Pre- immune control
<i>Controls</i>					Formalin fixed adrenals					
S-73§342 ^c (b)	f	cerebral haemorrhage; preterm	27	970	—	—	±	+++	—	—
S-73/396	m	RDS	29	1,160	±	—	+	++	—	—
S-83/380 ^c	m	IUD/placental insufficiency	31	1,180	+	++	±	+	—	—
S-84/003 ^c (b)	f	asphyxia	36	1,650	+	++	++	+	—	—
S-73/490	f	pneumonia	40	3,680	—	—	±	++	—	—
S-83/376 ^c	f	right heart failure IUD/left cardiac hypoplasia	40	4,300	++	++	—	+	—	—
S-73/406	f	cord prolapse	41	2,500	±	—	+	++	++	—
<i>Anencephalics</i>										
S-81/131	f		34	940	±	±	±	±	+	—
S-78/417	f		36	2,010	±	+	±	—	±	—
S-71/213	m		38	2,500	±	++	+	++	—	—
S-84/100 ^c (b)	f		38	2,100	++	—	++	+	—	—
S-80/276	f		39	2,740	—	—	—	—	—	—
<i>Controls</i>					Glutaraldehyde paraformaldehyde fixed adrenals					
S-83/380 ^c	m	IUD/placental insufficiency	31	1,180	+	—	—	+	—	—
S-84/003 ^c	f	asphyxia	36	1,650	+	++	±	+	+	—
S-83/376 ^c	f	IUD/left cardiac hypoplasia	40	4,300	+	+	—	?	—	—
<i>Anencephalic</i>										
S-84/100 ^c (b)	f		38	2,100	+	++	±	+	±	—

^a rounded off to the nearest whole week^b born alive; RDS = respiratory distress syndrome; IUD = intrauterine death^c obtained by HPO – not stored pre- or postembedding; ? cannot be judged (high background)

§ year in which adrenal was obtained

FC = fetal cortex; NSN = nicotin-stimulated neurophysin; DC = definitive cortex; GP = glycopeptide; OXT = oxytocin; AVP = vasopressin

In addition, adrenals of fetal rats were similarly collected from a litter of which five fetuses had been injected i.p. with 0.35 µg colchicin in 50 µg physiological saline on day 19 and five had been sham-injected with the vehicle only.

The fetal brains of two litters were alternatively either removed by brain aspiration or sham operated on day 19 of gestation. The adrenals of the surviving fetuses (seven out of nine of the experimental anencephalics and seven out of nine of the sham operated fetuses) were used on day 21 for radioimmunoassay (for details on the operating procedure see Swaab and Honnebie 1973; Honnebie and Swaab 1974).

Radioimmunoassay. Fetal adrenals from the rat were immediately put into 0.1 N HCl, while the human samples were kept at –25° C until extracted into 0.1 N HCl. The samples were sonicated and neutralised, after which they were extracted by means of Vycor glass powder and assayed for OXT and AVP by highly specific radio-immunoassays as described previously (Dogterom et al. 1980; Swaab and Oosterbaan 1983).

Staining procedure of human paraffin material. 1. Paraffin sections (6 µm) were cut, mounted on chrom-alum coated object slides,

deparaffinised, and hydrated. The procedure took place at room temperature unless stated otherwise.

2. They were rinsed three times in Tris buffered saline (TBS, 50 mM Tris/HCl, 0.9% NaCl, pH 7.6) for 10 min.

3. The first antibody was dissolved in TBS containing 0.5% triton-X-100. The following first antibodies were used: anti-vasopressin (W1 or Hilbert), anti-oxytocin (0-1-V), and non-immune serum (Rob or Ducky) diluted 1:500–1:1000 in TBS (for characteristics of these antibodies see Ravid et al. 1985). Antibodies against OXT-neurophysin and AVP-neurophysin, i.e., respectively, anti-estrogen stimulated neurophysin (anti-ESN 2C (9/4/73)) and anti-nicotine stimulated neurophysin (anti-NSN 1D (5/12/72)) (cf. Antunes et al. 1979) were used in dilutions of 1:500–1000 and 1:500–4000 respectively. These antibodies were kindly donated by Dr. A.G. Robinson, Pittsburg, USA. In addition, an antibody raised against the C-terminal glycopeptide part of the AVP precursor (anti-GP, donated by Dr. D. Smyth, London, U.K.) and an auto-antibody of a multiple sclerosis patient Sonja (auto-MS) (Hansen et al. 1983; Møller et al. 1985) probably recognising OXT-neurophysin, in a dilution of 1:200–500. The sections were incubated for 24–48 h at 4° C.

4. Rinsed in TBS (three times 10 min), incubated with goat-anti

rabbit (GAR) (Betsy 1:50) for 60 min, rinsed in TBS (3 times 10 min), incubated with peroxidase-antiperoxidase (PAP) 1:800 (60 min), rinsed in TBS (2 times 10 min) and subsequently in 0.05 M Tris-HCL buffer pH 7.6 for 5 min.

5. The DAB reaction 0.5 mg/ml 3-3'-diamino benzidine (DAB Sigma) in 0.05 M Tris/HCl containing 0.01% H₂O₂ (20 min) was stopped by rinsing in aqua dest, after which the sections were dehydrated and embedded in Entellan.

The procedure using auto-MS in a dilution of 1:500 followed the procedure for the other antibodies up to the GAR step instead of which the sections were incubated with a sheep anti-human IgG-HRP conjugate (prepared according to Boorsma and Streeter 1979) for 90 min, rinsed with TBS (3 times for 10 min) and incubated with DAB for 20 min. The DAB reaction was stopped by rinsing in Tris HCl 0.05 M and the sections were further treated as described above.

Characterisation of antibodies. For the characterization of anti-AVP and anti-OXT see reference mentioned above. In order to check the localization obtained with anti-AVP, an antibody against its neurophysin (anti-NSN) and against the glycopeptide part of the precursor (anti-GP) were applied. Anti-GP raised against bovine GP also bound to human GP (kindly donated by Dr. N.G. Seidah to Dr. F.W. van Leeuwen). Both anti-NSN and anti-GP stained identically in formalin-fixed human hypothalami. AVP cells in the supraoptic nucleus (SON), paraventricular nucleus (PVN) and suprachiasmatic nucleus (SCN) stained in an identical way, as has been described for anti-AVP (Fliers et al. 1985; Swaab et al. 1985). It was first tried to verify the results with anti-OXT using an antibody against its neurophysin (anti-ESN). However, since this antibody stained virtually all cells in the SON and also in the SCN, it most certainly also stained AVP cells (Fig. 1). Therefore, this antibody could not be used as a second, independent marker of OXT cells. Subsequently, for this purpose we used the auto-antibody of a multiple sclerosis patient (auto-MS) that stained OXT cells in the brain, as appeared from the following observations;

1. Auto-MS stained the OXT cells and not the AVP cells in the rat brain, as appeared from stainings on 4% paraformaldehyde-perfused material. In the homozygous diabetes insipidus Brattleboro rat, which has a hereditary inability to synthesize AVP and its associated neurophysin, the cells containing OXT and its associated neurophysin were stained in exactly the same pattern as had been described by Vandesande and Dierickx (1976) (Fig. 2). In the normal Wistar rat, auto-MS also stained the OXT cells as appeared from (a) the preferential peripheral localization of the stained cells in the PVN (Vandesande and Dierickx 1976), the preferential dorsal localization of the stained cells in the SON (Van Leeuwen et al. 1976), the higher proportion of stained cells in the rostral part of the SON and PVN (Swaab et al. 1975a) and the absence of staining in the SCN (Swaab et al. 1975b). In 5% glutaraldehyde fixed tissue no staining was obtained with auto-MS.

2. Also the staining of auto-MS in formalin-fixed human hypothalami was typical of OXT cells (cf. Fliers et al. 1985; Swaab et al. 1985) in that it did not stain the SCN and the majority of the SON cells (Figs. 3 and 4). A few smaller cells were found to stain in the dorsal zone of the dorsolateral SON (Fig. 4). Immunoreactive cells were found in the medial part of the SON and in the accessory supraoptic nucleus. In the PVN staining was more lateral than found for the AVP staining. Biochemical characterization of auto-MS strongly suggests that it does not bind to OXT itself but rather to its associated neurophysin.

3. Auto-MS did not bind in RIA to labelled AVP or OXT within a dilution range of 1:500–1:200,000 (for procedure see Dogterom et al. 1980).

4. In immuno-electrofocusing (for procedure see Van der Sluis et al. 1983), auto-MS stained one major acetic band at pH 4.3 (the site of the neurophysins).

5. In SDS gel electrophoresis (Van der Sluis et al. 1985) auto-MS stained one major band of 16kD and two minor bands of 38kD and 54kD.

Results

Human paraffin material

Two staining patterns could be distinguished in the fetal adrenal; (a) that of the OXT-related antibodies and (b) that of the AVP-related antibodies.

a) Anti-OXT and auto-MS stained droplet-like structures in the cytoplasm of cells in the fetal zone of the adrenal cortex (Fig. 8).

b) Anti-AVP, anti-NSN and anti-GP stained structures in the external part of the definitive zone of the adrenal cortex. This staining was localized in fiber-like structures running in the connective tissue septa and around, as well as within the parenchyma cells (Figs. 5 and 6).

Staining of OXT and AVP was generally somewhere between weak and moderate, while at times antibodies against parts of the precursors (auto-MS or anti-NSN and anti-GP, respectively) stained the same structures much more intensely. However, anti-GP staining was often negative.

In anencephalics a similarly distinct staining pattern for OXT- and AVP-related (Fig. 7) antibodies was found as in the control fetuses.

Glutaraldehyde-paraformaldehyde fixation generally failed to improve staining intensity, while the localization of OXT- and AVP-related staining remained unaltered.

Anti-ESN was negative in most subjects. If it stained, it sometimes did so in an OXT-like way (fetal zone), sometimes in an AVP-like way (definitive zone). Since staining in the human hypothalamus also showed cross-reaction of this antibody with AVP cells, anti-ESN could not be used to distinguish between AVP- or OXT-containing structures.

c) Control serum was negative in all cases.

Radio-immunoassay data

The concentration of OXT and AVP in the fetal adrenals appeared to be low. In unoperated rats levels of 0.4 pg OXT/adrenal and <0.2 pg AVP/adrenal were found. In adrenals of five sham-injected fetuses values of 1.5 pg OXT/adrenal and <0.9 pg AVP/adrenal were found, while following colchicine treatment these figures were 2.9 pg OXT and 3.1 pg AVP/adrenal respectively (the detection limit being 1.2 pg and 0.9 pg respectively). In an extract of seven adrenals from anencephalic rat fetuses and a similar one from sham operated controls the levels were <0.54 pg/adrenal for OXT and <0.24 pg/adrenal for AVP.

Generally human neonatal adrenals revealed low OXT levels, the median being 19 pg/g. AVP was only detectable in four out of nine determined adrenals (cf. Table 2).

Discussion

The amounts of OXT and AVP as determined by RIA were very low, and often even below detection level. Values in the rat were in the pg/mg range (fetal adrenal weight being approximately 1 mg; e.g. Jost 1975), and in the human adrenal even in the pg/g range. The present human neonatal data agree with the literature both in the adult and in the fetal adrenal (Ang and Jenkins 1984; Nicholson et al. 1984). There is no literature available on the fetal rat. However, the low peptide levels and the very modest increase in these levels induced by colchicine did not appear to make the fetal rat adrenal a very favourable model for

Table 2. Concentration of vasopressin and oxytocin in human neonatal adrenals

Number	Gestation [weeks] ^a	AVP [pg/g adrenal]	OXT [pg/g adrenal]	Approximate time between death and freezing of adrenals [h]	Clinical details
S-125	28	<6.4	<26.8	17	acute IUD e.c.i.
S-160	31	<2.9	<12.0	>19	IUD; omphalocele; one umbilical cord artery
S-88*	32	8.5	19.7	>35	breach extraction asphyctic at birth
S-13	33	16	45	>48	IUD e.c.i.
S-61*	33	59	226	13	asphyctic at birth multiple congenital malformations
S-32	34	<5.0	18.8	?	abruptio placentae IUD e.c.i.
S-113	36	<2.8	<11.8	<5	IUD, placental insufficiency; partial abruptio placentae
S-79	40	6.9	17.2	24	suboptimal APCTG
S-107	40	<4.8	21.1	7	IUD; placental insufficiency cardiac ventricle septum defect

^a rounded off to the nearest whole week; IUD = intrauterine death; APCTG = ante partum cardiotocogram

* born alive; e.c.i. = e causa ignota; ? = unknown

human adrenal studies, and immunocytochemistry was therefore restricted to human tissue.

In spite of these low levels and the 12 years for which some of the tissue had been kept in paraffin blocks, good staining was obtained. The present paper shows that OXT and AVP are produced in distinct parts of the human neonatal adrenal. OXT that was characterised chemically in the human fetal adrenal (Nicholson et al. 1984) and adult adrenal (Ang and Jenkins 1984) was found to be localized mainly in cytoplasmatic droplets in the fetal zone cells, while AVP was found mainly in fibre-like structures between parenchyma cells in the definitive cortex, but some-

times also clearly within these cells. This could well be the representation of the same cells from which these fibres run in a neurite-like way into the septa.

That OXT and AVP in the fetal adrenal are not derived from the fetal brain, but rather produced by adrenal cells or released in the adrenal by nerve fibres is evident from the presence of their associated neurophysins and for AVP in addition to the glycopeptide (GP) part of the precursor. Neurophysins were measured by Nicholson et al. (1984) and Ang and Jenkins (1984), and stained by anti-NSN and auto-Ms in the present paper. In addition, sometimes staining was accomplished in the definitive cortex with anti-GP. The

Fig. 1. Anti-estrogen-stimulated neurophysin (anti-ESN), an antibody supposed to stain oxytocin-related neurophysin, stains neurons in the suprachiasmatic nucleus of the human hypothalamus. Neurons in this nucleus contain vasopressin and its related neurophysin, but no oxytocin cells (cf. Swaab et al. 1985). Since this antibody consequently crossreacted with vasopressin cells, it could not be used for further study (subject #81241, male, 19 years of age, formalin fixation). The bar represents 100 µm

Fig. 2. Auto-MS, an auto-antibody of a multiple sclerosis patient, probably recognizing oxytocin-related neurophysin, was incubated with brain sections of a Brattleboro rat that is deficient in vasopressin and its related neurophysin. Cells are stained in a configuration that is characteristic of oxytocin neurons, leaving the cells in the central part of the paraventricular nucleus (C) unstained (cf. Vandesande and Dierickx 1976). III = third ventricle. The small black dots are erythrocytes (e) staining because of pseudoperoxidase activity. The bar represents 100 µm (preparation by Dr. F.W. van Leeuwen)

Fig. 3. Human hypothalamus incubated with an antibody against vasopressin-related neurophysin (anti-NSN). A great majority of the cells in the supraoptic nucleus (SON) contains vasopressin, and stains with this antibody (subject #81012, female, 38 years of age, formalin fixation, O-C = optic chiasm, B = bloodvessels – the same vessels are visible in the next figure). The bar represents 100 µm

Fig. 4. Alternating section of the SON incubated with auto-MS (cf. Fig. 3). Note that most SON cells are visible but are not positively stained. Only a small number of oxytocin cells localized on a characteristic site in the dorsal medial part of the SON stain with this antibody. These cells are smaller than most vasopressin cells stained in Fig. 3 (cf. Fliers et al. 1985). (Subject #81012. O-C = optic chiasm, B = bloodvessels; compare last figure). The bar represents 100 µm

Fig. 5. Anti-nicotin stimulated neurophysin (anti-NSN), an antibody directed against vasopressin associated neurophysin, stains the outer zone of the definitive cortex in the adrenal of a control neonate (subject #S-73/396, male, 29 weeks of gestation, formalin fixation). The bar represents 100 µm

Fig. 6. Anti-vasopressin (Hilbert) staining fibre-like structures in the septa around the parenchyma cells in the definitive cortex of a control adrenal. (Subject #S-84/003, female, 36 weeks of gestation, formalin fixation). The bars represent 100 µm

Fig. 7. Anti-NSN staining of the definitive cortex in an anencephalic adrenal. (Subject #S-71/213, male, 38 weeks of gestation, formalin fixation). The bar represents 100 µm

Fig. 8. Anti-oxytocin staining droplets in the cells of the fetal zone of the adrenal cortex. (Subject #S-83/380, male, 31 weeks of gestation, glutaraldehyde – paraformaldehyde fixation). The bar represents 50 µm

presence of the same distinctive OXT and AVP staining patterns in adrenals of anencephalics also pleads in favour of peptide production in the adrenal itself, since anencephalics do not have a peptide containing neurohypophysis (Visser and Swaab 1979). The fact that AVP is present in the adrenal gland of the Brattleboro rat (Nussey et al. 1984), the brain of which is incapable – as a result of a hereditary defect – of producing AVP, also supports this option.

In the last few years it has become apparent that the adrenal is the site of production of various peptides, while peptide-containing fibres also innervated the adrenal. Thus, substance-P (Rathsack et al. 1983) and peptides of the opiomelanocortin family like Met- and Leu-enkephalin, β -endorphin, ACTH, α -MSH and γ -MSH (Kondo et al. 1984; Kilpatrick et al. 1980; Wilson et al. 1980, 1981; Lewis et al. 1980; Fleminger et al. 1983; Evans et al. 1983; Schultzberg et al. 1978) have been reported to be present.

The presence of AVP and OXT in the neonatal adrenal of course does not prove that the peptides present in the amniotic fluid are actually derived from this fetal organ. The circumstantial evidence that these levels increase under conditions of fetal stress and labour (see introduction) (Oosterbaan et al. 1985b) have to be followed up by measurements of these peptides in amniotic fluid following removal of the fetal adrenal in a suitable experimental animal. On the other hand, the fetal brain is not the main source of amniotic OXT, in view of its presence in this compartment in human and in experimental anencephalics (Swaab and Oosterbaan 1983). Another theoretical source is the placenta that was reported to contain OXT and AVP (Levina 1968; Fields et al. 1983). In the rat we could not obtain any evidence for the presence of these peptides in placenta or membranes by RIA or immunocytochemistry. Recently we found in a few anencephalics slightly higher (although low) OXT levels in the umbilical cord veins than in the artery (Oosterbaan et al. 1986), so that the placenta remains a possible source for circulating OXT (and AVP) as in the human, to be investigated.

The physiological implications of the presence of OXT and AVP in the fetal adrenals are at present not yet clear. The peptides might be involved in the stress induced changes of the adrenal (Oosterbaan et al. 1985b). Another "stress related" peptide, CRF, has been found in the bovine adrenal medulla (Hashimoto et al. 1984). The peptides that are produced in the adrenal might either be released into the bloodstream as hormones and be of importance e.g. in the process of labour, or act as local messengers, e.g. being involved in the regulation of the function of steroidogenesis (cf. Nicholson et al. 1984; Cheung 1984). The finding that the bovine adrenal medulla contains V1-type receptors for AVP (Antoni 1984), argues in favor of such a possibility. It is not yet clear how the AVP and OXT producing sites in the adrenal change with development. However, it is of interest to note that OXT bloodlevels in the adult rat do not change following hypophysectomy (Dogterom et al. 1977). This suggests it might be that circulating OXT does not originate predominantly from the brain, but e.g., from the adrenal. On the other hand, AVP from the adult adrenal probably does not give rise to high circulating bloodlevels, since, in spite of its presence in the Brattleboro adrenal (Nussey et al. 1984), this mutant has a diabetes insipidus. This suggests that the primary function of adrenal AVP in the adult might be in the adrenal itself. Also the opioid peptides might act locally (Ungar and Phillips 1983).

This means that peptides from the adrenal might act either as a neurohormone or as a local messenger in a similar way as has been found in the brain (Swaab 1982). A third possible way of action of AVP and OXT is within the cells of production. In this relation the possible coexistence of amines and peptides in the adrenal (Fleminger et al. 1983; Verhofstad and Jonsson 1983; Wilson et al. 1981; Viveros et al. 1980; Leboulanger et al. 1984) is a point of interest.

The presence of alternative sources for OXT and AVP makes clear at the moment that many data on the possible functions of OXT have to be reconsidered for methodological reasons. Until now it has been e.g. deduced from the normal mean gestation length in human and rat anencephalics that fetal OXT could not be essential for the initiation of labour (cf. Swaab et al. 1977). However, the possibility that OXT might under these conditions have an alternative source was not taken into account at all. Peptide production in the fetal adrenal might, in relation to labour, be of particular interest, since following fetal adrenalectomy, labour in sheep does not take place (Liggins et al. 1967).

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