0031-3998/90/2805-0469$02.00/0
PEDIATRIC RESEARCH

Vol. 28, No. 5, 1990
Printed in U.S.A.

Changes in Selected Brain Neurotransmitters and Their Metabolites in the Lamb after Thyroidectomy during the Last Two Trimesters of Gestation or the Early Neonatal Period

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ABSTRACT. To evaluate in a developmental context the effect of congenital hypothyroidism on concentrations of the neurotransmitters norepinephrine, dopamine, and serotonin (5HT) in selected brain areas of the ovine fetus, we studied the effect of thyroidectomy at three ages on the concentrations of these neurotransmitters and their major metabolites, homovanillic acid and 5-hydroxyindoleacetic acid. Fetuses underwent thyroidectomy at 90–95 or 105–115 d gestation (term = 147–150 d) or 1–5 d after birth. Approximately 25 d after thyroidectomy, at d 120–125 or 130–135 of gestation or 25–30 d after birth, respectively, the ewes were killed and fetal brains removed. Neurotransmitters and their metabolites were measured by HPLC with electrochemical detection. Thyroidectomy in the 2nd trimester increased 5HT in five brain areas: anterior hypothalamus, dorsal medial hypothalamus, pons, medulla, and cerebellum. Thyroidectomy in the 3rd trimester increased 5HT in the pons and medulla, increased norepinephrine in the dorsal medial hypothalamus and pons, and increased homovanillic acid in the posterior hypothalamus. Thyroidectomy in the newborn period decreased NE in the anterior hypothalamus, ventral medial hypothalamus, and midbrain, decreased 5-hydroxyindoleactic acid in the posterior hypothalamus, lateral hypothalamus, dorsal medial hypothalamus, and ventral medial hypothalamus, and decreased homovanillic acid in the dorsal medial hypothalamus and ventral medial hypothalamus. From these data we conclude the following: *1*) Hypothyroidism causes changes in neurotransmitter concentrations only in selected brain areas of the ovine fetus, rather than causing generalized and similar changes in all brain ares; and *2*) 5HT 5-hydroxyindoleacetic acid concentrations are affected more often than the other neurotransmitters evaluated, perhaps because the 5HT neurotransmitter system is developing at these times. (*Pediatr Res* 28: 469–472, 1990)

Abbreviations

AH, anterior hypothalamus
DMH, dorsomedial hypothalamus
LaH, lateral hypothalamus
PH, posterior hypothalamus
VMH, ventromedial hypothalamus
NE, norepinephrine
DA, dopamine
HVA, homovanillic acid
5HT, serotonin
HIAA, 5-hydroxyindoleacetic acid
NT, neurotransmitter
Tx2, thyroidectomy in the 2nd trimester
Tx3, thyroidectomy in the 3rd trimester
TxNB, thyroidectomy in the newborn period

Received February 19, 1990; accepted June 5, 1990.
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Supported in part by Grant HD 21075 from the National Institutes of Health and grants from The Medical Research Council of New Zealand and the Wellcome Trust.

Hypothyroidism has been shown to affect most aspects of brain development including DNA content, protein content, NT content, synaptic organization, and myelination (1–3). Defining the mechanism(s) that causes these effects has proven difficult, however. The effects of hypothyroidism have been described in several species in relation to gestational age rather than a physiologic maturational event, and species differ in the time of maturational events in the brain.

Available data from various mammalian species concerning the effect of fetal hypothyroidism on brain NT are not consistent and do not give a clear picture of the mechanism by which brain (or brain nuclei) concentrations of the various NT are affected by hypothyroidism (4–10). We have chosen to study an ovine model because of the large size of the fetus and its accessibility for *in utero* thyroidectomy as well as the large body of data indicating similarity between human and ovine fetal thyroid physiology (11). We have therefore designed experiments to test the following hypothesis: Thyroidectomy in the ovine fetus causes distinctive changes in brain NT concentrations that are dependent on the developmental state of the animal at the time of thyroidectomy. Data for the control animals have also been reported elsewhere (Richards GE, Gluckman PD, Ball K, Mannelli SC, Kalamaras J, unpublished observations).

MATERIALS AND METHODS

Animals. Romney ewes were mated to Suffolk rams on a known date to produce fetuses of known gestational age for our study. Pregnant ewes were brought from the breeding farm to the Developmental Physiology Laboratory, University of Auckland, a few days before surgery. All procedures were approved by the Animal Ethical Committee of the University of Auckland.

Surgery. After an overnight fast, the animals were anesthetized with i.v. aphaxalone and maintained with halothane/oxygen via

an endotracheal tube. Using sterile technique, the fetal head was delivered through a midline abdominal incision. The fetal thyroid was removed, the fetus was returned to the uterus, and the uterus and abdomen were closed. Newborn animals had thyroidectomies performed after a 6-h fast under halothane/oxygen anesthesia.

Protocol. Postoperatively, fetuses were allowed to develop undisturbed until they were killed. Newborns were returned to their mothers and allowed to grow without further intervention until they were killed. Each group of animals was hypothyroid for approximately 25 d, after which the ewe or newborn was killed by barbiturate overdose and the fetal or newborn brain removed and frozen at −80°C within 10–15 min of death.

Experimental groups. Tx2 ($n = 5$) animals underwent thyroidectomy at 90–95 d gestation and were killed at 120–125 d gestation. Tx3 ($n = 10$) animals underwent thyroidectomy at 105–115 d gestation and were killed at 133–137 d gestation. TxNB ($n = 5$) animals underwent thyroidectomy at 1–5 d after birth and were killed at 25–30 d of age.

Plasma thyroxine was less than 25 nmol/L in every thyroidectomized animal at the time of death. In another group of animals ($n = 6$) not included in this report, we have verified that plasma thyroxine falls to less than 25 nmol by 48 h after surgery. Thus, we are confident of the completeness of thyroidectomy and the period of time during which the animals were hypothyroid.

Controls. Control animals were either unoperated twins of thyroidectomy animals, or animals who had carotid artery and jugular vein catheter placement without thyroidectomy and without any other experimental intervention. The procedure for killing the ewe and collecting the fetal brains was the same in all groups. There were five control animals for the Tx2 group (120–125 d gestation), eight control animals for the Tx3 group (133–137 d gestation), and five control animals for the TxNB group (25–30 d of age). Plasma thyroxine at the time of death in control animals was 154 ± 10.9 nmol/L, a value comparable to that reported by others (11–13).

NT. NT and metabolites were measured by HPLC with electrochemical detection as previously described (14). This method quantifies NE, DA and its major metabolite HVA, and 5HT and its major metabolite HIAA. 3-Methoxy-4-hydroxyphenylethylene glycol, the major metabolite of NE, could be measured on some occasions, but the peak merged with the solvent front often enough that 3-methoxy-4-hydroxyphenylethylene glycol results were not reliable enough to report here. Brains were dissected while frozen into the following areas according to the atlas of Gluckman and Parsons (15): AH, PH, LaH, DMH, VMH, midbrain, pons, medulla, cerebrum, and cerebellum. Brain areas were never thawed before assay.

Thyroxine. Thyroxine was measured by RIA (16).

Statistics. Each thyroidectomy group was compared with its appropriate control group by unpaired two-tailed t test (17). Differences are considered to be significant when $p < 0.05$.

RESULTS

The effects of thyroidectomy in the developing sheep at three different ages are described in Tables 1–5. Of all the brain areas examined in the three thyroidectomy groups, 14% were significantly different from controls. Animals in the Tx2 group had no change in NE, DA, HVA, or HIAA, but 5HT was significantly increased in five brain areas (AH, DMH, pons, medulla, and cerebellum) when compared with controls. The Tx3 group had increased NE in the AH, DMH, and pons, no change in DA, increased HVA in the PH, increased 5HT in pons and medulla, and decreased HIAA in midbrain. When thyroidectomy was performed after birth (TxNB group), NE was decreased in the AH, VMH, and midbrain; DA was unchanged; HVA was decreased in the DMH and VMH; 5HT was unchanged; and HIAA decreased in four areas (PH, LaH, DMH, and VMH).

Of the 21 reported differences between controls and thyroidectomy animals, the majority (13 of 21) were observed in the 5HT/HIAA system. To view these changes in another perspective, 5HT was increased in 50% of the brain areas in the Tx2 group and 20% of the Tx3 group. HIAA, on the other hand, was increased in only 10% of the Tx2 group and 10% of the Tx3 group, but was decreased in 40% of the TxNB group. Thus, although the total number of changes was relatively small, the relative percentage of the brain affected was much larger for the 5HT/HIAA NT system.

DISCUSSION

We have demonstrated that the effects of thyroidectomy on NT concentration are not global changes that affect every brain area and every NT in the same manner throughout development. On the contrary, the effect of hypothyroidism is specific for brain area and NT, making it unlikely that these effects are simply the manifestation of decreased NT metabolism in all cells. Our findings expand the results of a study by Rastogi and Singhal (6) in a rat model, in which 5HT decreased and HIAA increased in several brain areas after ^{131}I administration on the day of birth, and the findings of Savard *et al.* (5) in rats treated with propylthiouracil for the first 42 d of life. In the latter study, the 5HT content of discrete nuclei rather than of larger brain areas was measured. 5HT was increased in 11 nuclei of the hypothyroid rats and HIAA was higher in 16 nuclei compared with controls. In a study of 100-d-old rats that had undergone surgical thyroidectomy 3 wk earlier, a decrease in 5HT accompanied by a decrease in DA and no change in NE was reported by Ito *et al.* (9). A decrease in NE with no change in DA or 5HT has also been demonstrated in rats given propylthiouracil for 15 or 30 d after birth (4). The same authors (8) also found decreased NE and DA in brain areas of rats given ^{131}I on the day of birth.

These cross-species comparisons are difficult to interpret and relate to hypothyroidism in humans because of differences in developmental timing in the brain with respect to birth: the rat undergoes relatively more postnatal differentiation than the human, whereas the sheep undergoes relatively more development in the prenatal period (2, 18). The choice of timing of thyroidectomy in our study was based on a heuristic assumption that the early part of the 3rd trimester in the sheep and early postnatal life in the human represent roughly equivalent stages of brain development. We chose for comparison a time before and after the period of maximal interest. In the hypothyroid neonatal rat, the reversibility of the changes in NT concentrations demonstrated after treatment beginning at postnatal d 5 but not at postnatal d 20 (8) suggests that in this species, the critical time period for thyroxine's effect on NT lies somewhere between d 5 and 20. Our studies cannot address at this time the issue of whether the changes in NT that we describe are reversible with thyroxine therapy.

There are a number of possible explanations for our findings. One hypothesis is that hypothyroidism produces a delay in the normal ontogeny of neural development by interfering with NT synthesis, which would be reflected in reduced concentrations of NT in certain brain areas. The increases in 5HT we observed in the Tx2 and Tx3 groups and in NE in the Tx3 group are difficult to reconcile with this hypothesis. An alternative hypothesis is that hypothyroidism affects NT concentrations by interfering with normal NT metabolism, rather than synthesis.

Increases in NT in the Tx2 and Tx3 groups are more compatible with this hypothesis, but the decreases observed in both NT and metabolites in the TxNB group do not easily fit a mechanism of action based solely on changes in the metabolic fate of a normally manufactured NT. Because neuronal ontogeny includes the development of the capacity both to synthesize and metabolize NT and these processes are usually closely synchronized, it is difficult to distinguish experimentally between these hypothetical effects of hypothyroidism. The difference in the

Table 1. *NE concentrations in ng/mg (mean ± SEM) in three groups of sheep undergoing thyroidectomy at different ages compared with controls**

	Control	Tx2	Control	Tx3	Control	TxNB
AH	0.57 ± 0.21	0.47 ± 0.05	0.50 ± 0.08	0.79 ± 0.15	1.31 ± 0.18	0.68 ± 0.10†
PH	0.35 ± 0.07	0.31 ± 0.06	0.26 ± 0.01	0.31 ± 0.02	0.53 ± 0.15	0.44 ± 0.05
LaH	0.60 ± 0.28	0.32 ± 0.03	0.40 ± 0.09	0.47 ± 0.09	0.91 ± 0.11	0.56 ± 0.11
DMH	0.42 ± 0.11	0.53 ± 0.03	0.39 ± 0.01	0.80 ± 0.15†	1.40 ± 0.23	0.73 ± 0.08
VMH	0.51 ± 0.17	0.45 ± 0.06	0.54 ± 0.10	0.56 ± 0.09	1.40 ± 0.14	0.89 ± 0.18†
Midbrain	0.25 ± 0.01	0.34 ± 0.05	0.31 ± 0.03	0.31 ± 0.02	0.76 ± 0.13	0.37 ± 0.02†
Pons	0.38 ± 0.07	0.47 ± 0.07	0.31 ± 0.04	0.50 ± 0.07†	0.43 ± 0.06	0.42 ± 0.04
Medulla	0.41 ± 0.12	0.41 ± 0.04	0.29 ± 0.02	0.51 ± 0.12	0.52 ± 0.09	0.36 ± 0.04
Cerebrum	0.25 ± 0.01	0.25 ± 0.01	0.27 ± 0.02	0.25 ± 0.01	0.26 ± 0.01	0.29 ± 0.01
Cerebellum	0.25 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	0.30 ± 0.02	0.28 ± 0.01

* Treatment and control groups are described in detail in Materials and Methods.
† $p < 0.05$.

Table 2. *DA concentrations in ng/mg (mean ± SEM) in three groups of sheep undergoing thyroidectomy at different ages compared with controls**

	Control	Tx2	Control	Tx3	Control	TxNB
AH	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.32 ± 0.04	0.26 ± 0.02	0.26 ± 0.02
PH	0.28 ± 0.03	0.27 ± 0.02	0.25 ± 0.01	0.40 ± 0.15	0.26 ± 0.01	0.25 ± 0.01
LaH	0.25 ± 0.01	0.35 ± 0.07	0.25 ± 0.01	0.28 ± 0.04	0.29 ± 0.03	0.26 ± 0.01
DMH	0.25 ± 0.01	0.43 ± 0.11	0.27 ± 0.02	0.27 ± 0.02	0.27 ± 0.01	0.26 ± 0.01
VMH	0.26 ± 0.01	0.42 ± 0.12	0.25 ± 0.01	0.28 ± 0.03	0.31 ± 0.02	0.26 ± 0.01
Midbrain	0.26 ± 0.01	0.41 ± 0.07	0.27 ± 0.02	0.29 ± 0.04	0.29 ± 0.03	0.25 ± 0.01
Pons	0.25 ± 0.01	0.36 ± 0.07	0.25 ± 0.01	0.31 ± 0.03	0.26 ± 0.01	0.26 ± 0.01
Medulla	0.29 ± 0.04	0.36 ± 0.07	0.31 ± 0.06	0.28 ± 0.03	0.29 ± 0.01	0.25 ± 0.01
Cerebrum	0.25 ± 0.01	0.38 ± 0.08	0.25 ± 0.01	0.27 ± 0.02	0.25 ± 0.01	0.25 ± 0.01
Cerebellum	0.25 ± 0.01	0.36 ± 0.07	0.25 ± 0.01	0.44 ± 0.18	0.25 ± 0.01	0.25 ± 0.01

* Treatment and control groups are described in detail in Materials and Methods.

Table 3. *HVA concentrations in ng/mg (mean ± SEM) in three groups of sheep undergoing thyroidectomy at different ages compared with controls**

	Control	Tx2	Control	Tx3	Control	TxNB
AH	0.51 ± 0.13	0.47 ± 0.11	0.55 ± 0.12	0.98 ± 0.18	0.64 ± 0.12	0.38 ± 0.06
PH	0.39 ± 0.05	0.43 ± 0.09	0.28 ± 0.01	0.73 ± 0.14†	0.51 ± 0.06	0.34 ± 0.04
LaH	0.49 ± 0.10	0.50 ± 0.03	0.56 ± 0.01	1.03 ± 0.30	0.67 ± 0.10	0.39 ± 0.05
DMH	0.59 ± 0.15	0.54 ± 0.14	0.55 ± 0.09	0.81 ± 0.16	0.82 ± 0.07	0.37 ± 0.04†
VMH	0.51 ± 0.13	0.36 ± 0.07	0.55 ± 0.12	0.77 ± 0.21	0.69 ± 0.07	0.36 ± 0.04†
Midbrain	0.88 ± 0.19	0.62 ± 0.18	0.94 ± 0.03	0.90 ± 0.16	0.73 ± 0.15	0.55 ± 0.05
Pons	0.48 ± 0.11	0.36 ± 0.06	0.64 ± 0.19	0.70 ± 0.14	0.29 ± 0.12	0.39 ± 0.02
Medulla	0.47 ± 0.11	0.34 ± 0.10	0.32 ± 0.04	0.33 ± 0.03	0.31 ± 0.05	0.25 ± 0.01
Cerebrum	0.51 ± 0.14	0.25 ± 0.01	0.31 ± 0.05	0.26 ± 0.01	0.26 ± 0.01	0.25 ± 0.01
Cerebellum	0.41 ± 0.10	0.27 ± 0.02	0.26 ± 0.01	0.32 ± 0.05	0.25 ± 0.01	0.26 ± 0.01

* Treatment and control groups are described in detail in Materials and Methods.
† $p < 0.05$.

Table 4. *5HT concentrations in ng/mg (mean ± SEM) in three groups of sheep undergoing thyroidectomy at different ages compared with controls**

	Control	Tx2	Control	Tx3	Control	TxNB
AH	0.25 ± 0.01	0.51 ± 0.09†	0.35 ± 0.03	0.59 ± 0.17	0.51 ± 0.10	0.32 ± 0.04
PH	0.34 ± 0.06	0.53 ± 0.07	0.44 ± 0.07	0.53 ± 0.12	0.51 ± 0.01	0.32 ± 0.03
LaH	0.89 ± 0.32	0.52 ± 0.04	0.45 ± 0.11	0.64 ± 0.13	0.65 ± 0.23	0.37 ± 0.04
DMH	0.32 ± 0.06	0.69 ± 0.06†	0.53 ± 0.18	0.47 ± 0.08	0.63 ± 0.16	0.29 ± 0.01
VMH	0.42 ± 0.08	0.54 ± 0.07	0.38 ± 0.07	0.58 ± 0.11	0.82 ± 0.23	0.34 ± 0.04
Midbrain	0.94 ± 0.37	1.16 ± 0.16	0.68 ± 0.16	0.75 ± 0.11	0.84 ± 0.24	0.43 ± 0.05
Pons	0.51 ± 0.11	0.93 ± 0.03†	0.40 ± 0.05	1.24 ± 0.17†	0.61 ± 0.21	0.41 ± 0.03
Medulla	0.68 ± 0.12	1.10 ± 0.05†	0.43 ± 0.07	0.88 ± 0.16†	0.64 ± 0.21	0.32 ± 0.01
Cerebrum	0.29 ± 0.03	0.32 ± 0.05	0.33 ± 0.06	0.32 ± 0.05	0.62 ± 0.26	0.31 ± 0.02
Cerebellum	0.45 ± 0.11	1.03 ± 0.17†	0.59 ± 0.27	0.65 ± 0.18	0.42 ± 0.07	0.26 ± 0.01

* Treatment and control groups are described in detail in Materials and Methods.
† $p < 0.05$.

Table 5. *HIAA concentrations in ng/mg (mean ± SEM) in three groups of sheep undergoing thyroidectomy at different ages compared with controls**

	Control	Tx2	Control	Tx3	Control	TxNB
AH	0.51 ± 0.13	0.56 ± 0.10	0.73 ± 0.11	0.77 ± 0.10	0.71 ± 0.14	0.50 ± 0.11
PH	0.64 ± 0.24	0.60 ± 0.09	0.66 ± 0.09	0.71 ± 0.09	0.77 ± 0.12	0.36 ± 0.03†
LaH	0.58 ± 0.10	0.53 ± 0.06	0.79 ± 0.10	1.03 ± 0.15	0.79 ± 0.09	0.40 ± 0.08†
DMH	0.62 ± 0.16	0.70 ± 0.06	0.70 ± 0.12	0.79 ± 0.09	1.13 ± 0.11	0.40 ± 0.03†
VMH	0.67 ± 0.24	0.49 ± 0.05	0.78 ± 0.10	0.79 ± 0.08	1.04 ± 0.05	0.49 ± 0.05†
Midbrain	1.19 ± 0.59	1.07 ± 0.22	1.60 ± 0.31	0.93 ± 0.08†	1.35 ± 0.38	0.96 ± 0.14
Pons	0.86 ± 0.11	1.94 ± 0.19†	1.49 ± 0.21	1.33 ± 0.20	1.34 ± 0.70	0.76 ± 0.09
Medulla	1.23 ± 0.31	1.43 ± 0.17	0.91 ± 0.20	0.80 ± 0.07	1.14 ± 0.18	0.78 ± 0.21
Cerebrum	0.59 ± 0.16	0.25 ± 0.01	0.41 ± 0.08	0.36 ± 0.05	0.42 ± 0.07	0.31 ± 0.02
Cerebellum	0.55 ± 0.18	0.36 ± 0.11	0.35 ± 0.06	0.60 ± 0.13	0.37 ± 0.06	0.35 ± 0.04

* Treatment and control groups are described in detail in Materials and Methods.
† $p < 0.05$.

direction of change of NT concentration when thyroidectomy occurs before *versus* after birth suggests that the mechanisms underlying these changes may be different at these two times.

Our study did not attempt to identify the specific mechanism by which thyroidectomy led to changes in NT concentrations, and our results could be interpreted as consistent with either hypothesis. In the lamb undergoing normal ontogeny, 5HT concentrations in early gestation have not been evaluated, but they remain stable while HIAA concentrations increase from 120–125 d through the newborn period (14; Richards GE, Gluckman PD, Ball K, Mannelli SC, Kalamaras J, unpublished observations). Our observation that HIAA concentrations decrease during this same period in the thyroidectomized fetus could be interpreted to suggest that the ontogeny of 5HT synthesis is arrested as a consequence of hypothyroidism.

In summary, our studies have demonstrated that in our fetal ovine model, hypothyroidism changes NT in several brain areas and the direction of these changes is consistent in its dependence on the stage of development of the animal. The specificity of these changes with respect to brain area and NT involved indicates that detailed studies of the synthesis and turnover of multiple NT will be necessary to clarify the mechanism of the changes we have observed.

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