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Development of Regulatory Mechanisms in the Thyroid: Failure of Iodide to Suppress Iodide Transport Activity (41375)

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Abstract. Fetal, neonatal, and adult cat thyroid glands were investigated for their ability to respond to excess iodide *in vitro* with reduction in both iodide transport activity as measured by the ^{125}I-T/M ratio and thyrotropin-stimulated cyclic AMP formation. Fetal and neonatal cat thyroids responded to thyrotropin addition with greater increases in cyclic AMP than those of adults. Moreover, in each group, pretreatment of the thyroid tissue with NaI (30 μmol for 2 hr) resulted in a significant reduction in the subsequent stimulation of cyclic AMP formation by thyrotropin. The ^{125}I-T/M ratio in neonatal cat thyroid tissue was similar to that in adult animals of either sex, whereas the ^{125}I-T/M of fetal thyroid was greater than that of the mother. Moreover, while pretreatment of the thyroid tissue of neonatal or adult animals with excess iodide resulted in a reduction in iodide transport activity, no such reduction was observed in fetal thyroid tissue. These findings suggest that the autoregulatory effect of iodide transport develops at a later gestational time than the action on thyrotropin-stimulated cyclic AMP formation.

Exposure of thyroid tissue to high concentrations (5–50 μM) of iodide *in vitro* results in a reduction in subsequently determined iodide transport activity (1–3), as well as an inhibition of the stimulation of cyclic AMP formation induced by thyrotropin (TSH) or other thyroid stimulators (2, 4–9). These two actions of iodide on thyroid function are similar in that both are prevented by simultaneous treatment of the tissue with agents that inhibit intrathyroidal oxidative reactions, and both effects appear to occur through the mediation of as yet unidentified, iodide-containing inhibitory factors formed within the thyroid cell (1, 4). There is no information available to indicate whether these two actions of iodide are mediated by the same or different iodide-containing inhibitory factors. The present study was undertaken to (a) ascertain whether these two actions of iodide can be observed in fetal or neonatal thyroid tissue and (b) to determine whether or not there is any difference in the fetal development of the thyroid responses to excess iodide.

Material and Methods. A description of the animal groups employed in this study is presented in Table I. All animals were obtained from a local supplier (Quaker Farms, Quakertown, Pa.). They were housed in environmentally controlled rooms and allowed food and water *ad libitum*. In those groups utilizing newborn animals, the female was allowed to deliver normally and the date of delivery recorded during routine daily observation. Date of birth was taken as day zero. Pregnant and lactating groups were composed of animals donating the fetal and neonatal groups, respectively. Fetal and neonatal thyroids were obtained by careful dissection, weighed to the nearest 0.1 mg on a torsion balance, and sectioned into eight pieces by dividing each lobe along its vertical and horizontal axis. Four thyroid sections, two from each lobe, were preincubated for 2 hr in 2.5 ml Eagle's minimum essential medium (10) supplemented with 30 μM NaI. The remaining sections were preincubated for 2 hr in the same medium without added iodide. Following the preincubation, the sections were subjected to two 30-min washes with Eagle's medium prior to the test incubation. This wash procedure has been shown previously to effectively remove more than 90% of the added iodide (3). Finally, two thyroid sections from each preincubation group were taken for measurement of iodide transport activity and the remaining sections employed for the

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TABLE I. DESCRIPTION OF EXPERIMENTAL GROUPS

Group	Litters	Animals	C–R length[a] (mm)	Weight (g)
I. Fetal, 40–45 days	3	10	87 ± 4	45 ± 6
II. Fetal, 55–60 days	5	19	121 ± 2	92 ± 3
III. Neonates, 1–3 days	4	16	138 ± 6	107 ± 10
IV. Neonates, 5–10 days	3	10	—	184 ± 14
V. Pregnant females	—	8		3150 ± 250
VI. Lactating females	—	5		2830 ± 130
VII. Normal females	—	4		2720 ± 150
VIII. Normal males		8		2640 ± 230

Note. Values given represent the mean ± SE of the measurements made in all animals within the experimental group.

[a] Crown to rump length.

measurement of TSH-stimulated cyclic AMP formation. Adult cat thyroid tissue was sliced with a Stadie-Riggs tissue slicer, further divided into sections with a razor blade, and subjected to the same preincubation, wash, and incubation procedure described for the fetal and neonatal groups.

The procedures employed for measurement of the ^{125}I-T/M ratio and cyclic AMP formation have been described previously (2, 3). Briefly, iodide transport activity of thyroid tissue was measured following a 90-min incubation in Eagle's medium (10) containing 1 μM NaI, as well as 3 mM methimazole and supplemented with 0.2 μCi/ml ^{125}I (New England Nuclear). TSH-stimulated cyclic AMP accumulation in cat thyroid tissue was measured following a 20-min incubation in Eagle's medium (10) containing 0.5% bovine serum albumin (Sigma) and 3 mU/ml TSH (NIH B-8). Cyclic AMP was measured by a modified protein kinase method as described previously (2).

Results. *Action of iodide on TSH-stimulated cyclic AMP formation.* As shown in Table II, preincubation of fetal, neonatal, or adult thyroid tissue for 2 hr in the presence of 30 μM NaI resulted in a reduction in the subsequent TSH stimulation of cyclic AMP accumulation. The inhibitory effect of iodide pretreatment was between 30 and 48% and was similar in all experimental groups. Addition of 3 mU/ml TSH to fetal and neonatal thyroid tissue resulted in an increase in cyclic AMP levels to values that were two- to fourfold greater than those observed in adult tissue. While

TABLE II. INHIBITION OF TSH-STIMULATED cAMP FORMATION BY IODIDE PRETREATMENT

Group	TSH-stimulated cAMP (pmol/mg)		Percentage change
	2-hr control preincubation	2-hr 30 μM NaI preincubation	
I. Fetal	93.5 ± 11.3	64.4 ± 8.1*	−30 ± 6
II. Fetal	68.5 ± 12.5	33.6 ± 9.0*	−44 ± 9
III. Neonate	123 ± 12.3	75.3 ± 8.8*	−33 ± 9
IV. Neonate	58.5 ± 11.5	39.3 ± 7.5*	−30 ± 6
V. Pregnant females	28.4 ± 6.5	13.9 ± 3.0*	−42 ± 6
VI. Lactating females	30.5 ± 8.8	15.5 ± 4.2*	−46 ± 8
VII. Normal females	28.3 ± 10.0	18.9 ± 5.8*	−34 ± 4
VIII. Normal males	32.4 ± 8.4	16.8 ± 5.6*	−48 ± 7

Note. Descriptions of the various experimental groups are summarized in Table I. Thyroid tissue was preincubated 2 hr in the presence or absence of 30 μM NaI, subjected to two 30-min washes to remove excess iodide, then incubated for 30 min for determination of cAMP formation. TSH (3 mU/ml) was added to all flasks during the final 15 min of the test incubation. Values are means ± SE.

*$P < 0.05$ by paired t test in comparison with tissue preincubated in the absence of added iodide.

TABLE III. INHIBITION OF THYROID IODIDE TRANSPORT BY IODIDE PRETREATMENT

Group	^{125}I-T/M		Percentage change
	2-hr control preincubation	2-hr 30 μM NaI preincubation	
I. Fetal	27.4 ± 5.5	23.3 ± 3.1	+18 ± 32
II. Fetal	28.3 ± 2.2	23.2 ± 2.4	−15 ± 10
III. Neonate	27.7 ± 3.9	13.8 ± 2.2*	−40 ± 6
IV. Neonate	25.1 ± 2.5	17.8 ± 1.1*	−25 ± 4
V. Pregnant females	24.4 ± 5.7	13.9 ± 2.6*	−41 ± 4
VI. Lactating females	30.6 ± 8.6	15.3 ± 4.3*	−48 ± 7
VII. Normal females	37.3 ± 9.1	18.6 ± 8.2*	−52 ± 8
VIII. Normal males	32.4 ± 5.4	16.6 ± 3.5*	−52 ± 7

Note. Descriptions of the various experimental groups are summarized in Table I. Thyroid tissue was preincubated for 2 hr in the presence or absence of 30 μM NaI. The tissue was subjected to two 30-min washes to remove excess iodide, then incubated for 60 min in medium containing 1.0 μM NaI supplemented with 0.2 μCi/ml ^{125}I for determination of the ^{125}I-T/M ratio. Values are means ± SE.

*$P < 0.05$ by paired t test comparison with tissue preincubated in the absence of added iodide.

there was insufficient fetal tissue to determine the basal, or unstimulated level of cyclic AMP in all animals, such measurements in four animals of group II revealed values of 2.3–4.6 pmole/mg. These were not significantly higher than the control level of cyclic AMP observed in either the maternal or other adult thyroids (data not shown, see Ref. (3)).

Action of iodide on thyroid iodide transport. The ^{125}I-T/M ratios obtained in fetal and neonatal thyroid tissue, preincubated in the absence of added iodide, were between 25 and 28 (Table III). These values were not significantly different from the values obtained in the various groups of adult animals. As previously reported, pretreatment of adult feline thyroid tissue for 2 hr with 30 μM NaI resulted in a 40 to 50% inhibition in the subsequently determined ^{125}I-T/M ratio. A similar inhibitory effect of excess iodide was observed in both the newborn cats (Group III) and in the 5- to 10-day-old kittens (Group IV). In contrast to these findings, pretreatment of fetal thyroid tissue with excess iodide failed to elicit a significant reduction in iodide concentrating activity. While some individual fetal thyroids responded to excess iodide with a reduction in iodide transport, others showed no response or an increase in iodide concentrating activity. As shown in Fig. 1, the ^{125}I-T/M ratios obtained in fetal tissue were higher than the maternal values. The fetal ^{125}I-T/M:maternal ^{125}I-T/M ratio was 1.45 ± 0.19 (SE), which was significantly ($P < 0.05$ by t test comparison) higher than the value of 0.95 ± 0.08 (SE) obtained in comparing the newborn:maternal transport ratios. Moreover, there was no apparent relationship between the fetal age and the presence or absence of an inhibitory effect of excess iodide.

Discussion. While fetal thyroid tissue is known to synthesize and secrete thyroxine in response to endogenous and exogenous TSH (11), there is little information available on the development of the control mechanisms involved in the regulation of thyroid cell activity. Studies in the fetal sheep indicate that the fetal thyroid secretes as much as four times the amount of thyroxine than the adult on a body surface area basis (11). This suggests a high level of thyroid gland activity. However, TSH concentrations in fetal serum are generally lower than maternal values during the later part of gestation. This indicates that the fetal thyroid is more sensitive to TSH stimulation. This conclusion is supported by our present findings that the fetal thyroid responds to TSH treatment with an increase in cyclic AMP levels that are two to three times that observed in the adult.

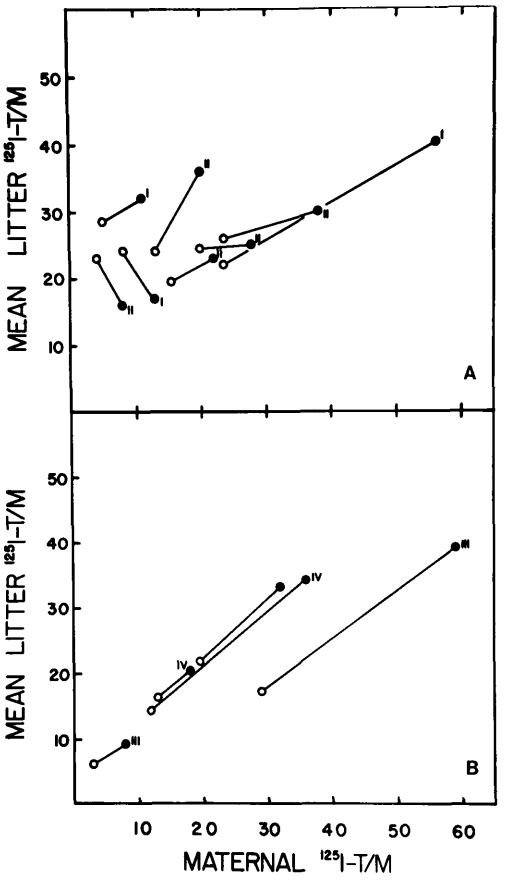

FIG. 1. Relationship between the iodide concentrating activity of fetal (A) or neonatal (B) thyroid and that of the corresponding maternal thyroid tissue. Closed circles represent values obtained in tissue preincubated in the absence of iodide whereas open circles represent the values obtained following pretreatment for 2 hr with 30 μM NaI. The roman numerals (I–IV) represent the experimental groups shown in Table I.

However, it is possible that the increased sensitivity of the fetal thyroid to TSH, as measured by increased cyclic AMP formation, might be the consequence of increased cellularity (or reduced colloid content) of the fetal tissue, or an increased sensitivity of the fetal thyroid due to reduced *in vivo* TSH stimulation and subsequent reduced desensitization to TSH. Neither of these possibilities can be excluded by the present study and will require further investigations. However, our studies indicate that this greater responsivity of the thyroid to TSH is maintained at least during the early postpartum period and the observation that fetal thyroid tissue responds to excess iodide with a suppression of TSH-stimulated cyclic AMP formation suggests that this response develops early in thyroid gland development.

Studies on the development of thyroid gland function have indicated that thyroid iodide transport, or the more commonly measured index, iodide incorporation into protein, appear simultaneously with the development of the thyroid follicle (11–15). The present finding demonstrates that iodide transport activity is well developed by the final third of the gestational period in the cat. Mean fetal litter iodide transport activity is higher than maternal, with the mean litter ^{125}I-T/M to maternal ^{125}I-T/M ratio being about 1.5. Similar values have been obtained by indirect measures of fetal thyroid activity in the macaque monkey (13) and humans (16). These observations suggest that the fetal thyroid is more active than the maternal thyroid. However, the degree of increased iodide transport observed in the fetus is not of the magnitude of the observed increased secretion of thyroid hormone (11). Moreover, in contrast to the newborn or adult thyroids, this activity was not consistently depressed by exposure to high iodide concentrations. The failure to observe this action of iodide in fetal tissue is unexplained. It is possible that the fetal thyroid does not possess the necessary oxidative enzymes necessary for the iodination of the iodide-containing inhibitory factor responsible for suppressing iodide transport. However, this seems unlikely, since the fetal thyroid responds to excess iodide with a reduction in TSH-stimulated cyclic AMP formation, an effect of iodide which also appears to be mediated through the action of an iodine-containing inhibitory factor (4–9). It is possible that the absence of an iodide-induced reduction in fetal iodide transport activity is the consequence of either a lack of the synthesis of the transport-related, iodine-containing, inhibitory factor precursor at this stage of development, or that the iodination of this factor is mediated through a separate iodin-

ation pathway than that utilized for the action of iodide on TSH-stimulated cyclic AMP. In either case, the present findings provide support for the presence of separate pathways mediating the inhibitory effects of excess iodide on iodide transport and TSH-stimulated cyclic AMP accumulation.

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