Maternal–Fetal Endocrine Interrelations in the Rat: Effects of Cyclic and Dibutyryl Cyclic AMP on Pituitary–Thyroid and Adrenocortical Systems in Mother and Neonate (37446)

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There is now persuasive evidence that 3′,5′-cyclic adenosine monophosphate (cyclic AMP, CAMP) is the intracellular mediator for a large variety of hormonal effects (1–3). Recent *in vivo* and *in vitro* studies utilizing cyclic AMP or its dibutyryl derivative (db CAMP) have shown that these nucleotides exert a strong influence on morphologic and functional parameters of endocrine gland secretion. Hormonogenesis in rat adrenals is enhanced with CAMP (4) and dbCAMP partially maintains weight, DNA, RNA and protein content of the adrenal gland following hypophysectomy (5). Marked effects of cyclic AMP and dbCAMP on thyroid gland metabolism have also been described. These nucleotides qualitatively mimic some of the primary actions of TSH on the thyroid. Cyclic AMP stimulates organic binding and iodothyronine formation in the rat thyroid (6), and dbCAMP promotes thyroid hormone secretion in rat (7) and mouse (8, 9). The dibutyryl derivative of cyclic AMP also stimulates colloid droplet formation, glucose oxidation and ^{32}P incorporation in thyroid slices (10–12). Conversely, intracellular levels of cyclic AMP, colloid droplet formation and iodine release from the incubated mouse thyroid are all increased by TSH (13).

The *in vivo* responses of adrenal and thyroid to nucleotide treatment may result from rise in cyclic AMP levels and release of ACTH and TSH by the adenohypophysis. Cyclic AMP increases substantially in the rat pituitary after chronic adrenalectomy (14) or thyroidectomy (15). *In vitro* release of TSH and ACTH is enhanced with dbCAMP and potentiated by theophylline (14, 16). There is also suggestive evidence that hypophysiotrophic factors regulate the release of the adenohypophysial hormones by influencing the activity of the adenyl cyclase–CAMP-phosphodiesterase system in pituitary cells. TRH promotes rapid increase in cyclic AMP levels of the incubated rat pituitary; the rise precedes the augmentation in TSH release (15). Close correspondence is also observed between the onset of rise in pituitary CAMP and stimulation of TSH and growth hormone release with hypothalamic extracts (17).

The possibility that significant change in the adenyl cyclase–CAMP–phosphodiesterase cellular system is fundamentally associated with alterations in hormonal activity during pregnancy has received scant attention. It has been shown that the administration of goitrogens and adrenal inhibitors to the pregnant rat induced hypersecretion of TSH and ACTH by the fetal hypophysis (18), although exogenous cyclic AMP treatment did not modify appreciably pituitary–thyroid function in mother or neonate (19). A systematic study of the effects of nucleotides on maternal–fetal endocrine interrelations appears to be urgently needed. The present work was designed to test the applicability of the Sutherland–Robison (1) second messenger concept to those hormonal events characteristic of the rat perinatal period. Specifically, the effects of cyclic AMP and dbCAMP administration on the pituitary–thyroid and adrenocortical system of mother and neonate are described. Parallel observations on young and adult nonpregnant rats are also recorded.

Materials and Methods. Virgin female rats (CD strain obtained from Charles River Breeding Laboratories, Wilmington, MA)

were mated by the commercial supplier. Males were placed with females at 4 PM on the day of proestrus and removed the next morning (7 AM). The presence of sperm in the vagina was taken as an indication of pregnancy (first day). Timed-pregnant rats arrived at the laboratory on Days 14–16 of pregnancy and were kept in separate breeding cages under controlled conditions of temperature (23 ± 1°) and artificial illumination (7 AM–7 PM). Unrestricted amounts of tap water and Purina Laboratory Chow were allowed. The response of the pituitary–thyroid and pituitary–adrenocortical systems to 3′,5′-cyclic adenosine monophosphate (CAMP) and dibutyryl adenosine 3′,5′-cyclic monophosphate (dbCAMP) administration was tested in adult, nonpregnant rats (220–250 g) by sc injection. The nucleotides were dissolved in saline and were injected (10 mg) twice daily, for 4 consecutive days and autopsy was made 18–20 hr after the last injection. Pregnant rats received daily (25 mg) or twice daily (10 mg) injections of the nucleotides over the last week of gestation; controls received saline injections. Treatment was discontinued after spontaneous delivery occurred. Pregnant rats usually delivered on Days 21–22 of gestation. Pups of litters which were delivered overnight were designated as 0 hr old at 9:00 AM the next morning.

Thyroid radioiodine uptakes in mother and newborn were determined after ip administration of tracer doses of ^{131}I and ^{125}I. Nonpregnant rats were autopsied 23–24 hr after injection of 10–12 μCi of ^{125}I. Mothers received injections of 10 μCi of ^{131}I just after spontaneous delivery and were sacrificed 22–24 hr later. Their newborn were injected with 0.5 μCi ^{131}I and were autopsied at similar time intervals. Thyroid radioactivity in some newborn and for pups throughout the first postnatal week was measured 18–20 hr after ip injection of ^{125}I (0.5–1.0 μCi). Thyroid glands were homogenized in vials containing 2% NaOH and radioactivity was determined in a well-type scintillation counter. Methods of blood collection for corticosteroid, radioactivity and TSH determinations were standardized and similar to those described previously (18–20). Plasma was stored at −15° until ready for chemical or bioassay studies.

At autopsy, adenohypophyses, thyroids and adrenals were removed, weighed to the nearest 0.1 mg and prepared for assay or chemical study. Corticosteroid content of plasma and adrenal was determined by Guillemin's modification of the Silber fluorometric procedure (21). Residual fluorescence (1.8–2.5 μg/100 ml) was routinely subtracted from plasma values. Corticosteroid concentration of neonatal adrenals was determined on several pairs of pooled glands from offspring of each experimental group. TSH content of pooled plasma and acid saline extracts of pituitaries were bioassayed in the stasis tadpole (22). TSH potency and 95% confidence limits were obtained by subjecting the assay data to analysis of variance (23). The standard TSH dose–response curve was obtained with NIH bovine standards and a purified rat TSH preparation. The index of precision or lambda (λ) for 6 groups of TSH assays averaged 0.130. Endocrine gland weights were related to final body weight and are recorded herein as milligrams per 100 g body. Data were also statistically analyzed by Student's *t* test.

Results. Administration of dbCAMP to virgin rats induced significant alterations in the activity of the pituitary–thyroid system (Table I). Although weight of thyroid and adenohypophysis scarcely changed, thyroid gland radioactivity was significantly lowered while TSH concentrations in plasma and pituitary were increased approximately 2-fold. Corticosteroid content of the adrenal and weight of the gland remained in the normal range; plasma corticosteroid levels declined moderately after dbCAMP treatment.

Nucleotide administration during the last week of pregnancy failed to influence maternal endocrine gland weights. Thyroidal radioiodine uptake in mothers was consistently less than in virgin rats but was unaffected by CAMP or dbCAMP treatment (Table I). Administration of the latter substance, however, resulted in a significant elevation (2-fold) in maternal plasma TSH level and slight increase in pituitary TSH concentration. No significant effect of cyclic AMP on

TABLE I. Effects of Nucleotide Administration on the Pituitary–Thyroid and Adrenocortical Systems.

Treatment (no. rats)	Endocrine gland wt (mg/100 g body ± SE)			Thyroid radioactivity (%/mg ± SE)	Corticosteroid		TSH assay (95% conf. limits)	
	Pituitary	Thyroid	Adrenal		Plasma (μg/100 ml ± SE)	Adrenal (μg/g ± SE)	Plasma (mU/100 ml)	Pituitary (mU/mg)
Nonpregnant[a]								
Normal (17)	3.8 ± 0.3	6.3 ± 0.4	26.7 ± 0.6	0.69 ± 0.08	52.5 ± 5.6	37.1 ± 5.0	56 (48– 70)	22.1 (18.7–26.1)
dbCAMP (16)	3.4 ± 0.2	6.4 ± 0.3	28.5 ± 1.2	0.48 ± 0.06[d]	30.3 ± 4.7[d]	39.7 ± 4.3	101 (81–125)[d]	40.2 (33.7–47.9)[d]
Mothers[b]								
Normal (9)	3.1 ± 0.2	5.2 ± 0.3	23.8 ± 1.1	0.27 ± 0.02	31.6 ± 6.5	47.5 ± 6.6	64 (51– 81)	22.4 (18.2–27.6)
dbCAMP (7)	3.4 ± 0.4	5.4 ± 0.7	23.8 ± 1.3	0.29 ± 0.06	24.0 ± 5.9	48.0 ± 5.3	150 (121–188)[d]	28.6 (22.3–35.3)
CAMP (5)	3.2 ± 0.2	4.8 ± 0.4	25.2 ± 3.2	0.31 ± 0.10	13.7 ± 3.7[d]	56.4 ± 6.8	70 (56– 87)	18.5 (14.9–23.3)
Newborn[c]								
Normal (122)	10.6 ± 0.9	14.4 ± 0.8	40.6 ± 2.0	6.7 ± 0.06	—	8.0 ± 1.2	67 (47– 95)	3.8 (2.7– 5.3)
dbCAMP (87)	10.0 ± 0.5	14.0 ± 0.7	39.1 ± 1.5	7.7 ± 0.36	—	11.1 ± 1.4	163 (119–232)[d]	7.3 (5.2–10.2)[d]
CAMP (50)	10.0 ± 0.6	15.6 ± 0.8	38.6 ± 1.3	7.3 ± 0.70	—	12.5 ± 1.4	85 (61–118)	3.7 (2.6– 5.2)

[a] Injected sc with 10 mg dbCAMP, twice daily, for 4 days; autopsy done 23–24 hr after ^{125}I injection.
[b] CAMP and dbCAMP, 25 mg daily, or 10 mg twice daily, over last 5 days of gestation.
[c] Newborn killed 2–24 hr after delivery and 22–24 hr after ^{131}I injection.
[d] Means differ significantly ($p = 0.05$ or less) from normal value.

TSH levels was observed. Response of the maternal pituitary–adrenocortical system to nucleotide treatment was minimal. Adrenal and plasma corticosteroid concentrations were highly variable. As in virgin rats, maternal plasma corticosteroid levels fell after administration of nucleotides, significantly so with CAMP.

Administration of cyclic AMP or dbCAMP to pregnant rats failed to alter body weight or endocrine gland weight of their newborn. Plasma and pituitary TSH levels in offspring of dbCAMP treated mothers were increased approximately 100% but remained within the normal range in newborn of mothers receiving CAMP. ^{131}I accumulation in the newborn's thyroid was not appreciably altered by nucleotide treatment during pregnancy. DbCAMP treatment of young rats during the first postnatal week resulted in significant stimulation of the pituitary-thyroid system (Table II). Radioiodine uptake by the thyroid gland normally declined sharply during the first postnatal week. Thyroidal radioactivity was reduced by 70% in 4 day old rats to reach still lower values by the end of the first postnatal week. Injection of the nucleotide resulted in 60–80% augmentation of thyroidal radioactivity at these time intervals. TSH levels in plasma and pituitary were also elevated and by the fifth postnatal day of dbCAMP treatment had increased approximately 50%. Adrenal gland weight–body weight ratio characteristically declined during the first postnatal week but this was unaffected by nucleotide treatment.

Discussion. The differential effects of nucleotide administration at high dose levels on pituitary–thyroid and adrenocortical systems in mothers and neonate are difficult to explain. It has already been demonstrated (18) that the fetal hypophysis has the capacity to augment ACTH and TSH secretion simultaneously in late gestation by appropriate treatment of the pregnant rat. Neither cyclic AMP nor its dibutyryl derivative produced any discernible stimulatory influence on the pituitary–adrenal system in these experiments. Aside from some reduction in plasma corticosteroid levels in virgin and pregnant rats, there was no indication from other adrenal parameters that any major change in ACTH secretion occurred. In contrast, impressive effects of dbCAMP but not cyclic AMP administration, were observed on the pituitary–thyroid system. Plasma and pituitary TSH levels of virgin and pregnant rats were consistently elevated after treatment with the nucleotide. Enhancement of TSH secretion was noted in both mother and newborn and was elicited also in offspring receiving dbCAMP injections throughout the first postnatal week. Radiometric changes in the thyroid were less consistent despite increased plasma TSH concentrations. Augmentation of TSH levels in nonpregnant rats was associated with reduced thyroidal radioactivity yet no significant changes in thyroidal radioiodine uptake were noted in mother and offspring despite increased TSH. On the other hand, elevation in TSH levels was coupled with increased thyroidal radioiodine uptake during the first postnatal week of dbCAMP treatment. The substantial effects of dbCAMP on TSH secretion and thyroid function, as against the ineffectiveness of cyclic AMP, may possibly be explained by greater penetrability into cells and resistance to intracellular enzymatic degradation of the former nucleotide. Recent studies, however, reveal that the ability of the dibutyryl derivative to simulate TSH-like effects on the thyroid, at least *in vitro,* may be due predominantly to intracellular activity of the substituted nucleotide itself (24). Studies on the *in vitro* adrenal also indicate that dbCAMP is more active than CAMP in promoting steroidogenesis even though the latter penetrates adrenal cells more rapidly than the dibutyryl derivative (27). Differences in transplacental movement and in penetration and intracellular activity of dbCAMP in thyroid, adrenal and anterior pituitary cells of mother and fetus may possibly account for the differential response of the pituitary–target gland systems noted in our *in vivo* study.

The *in vivo* action of dbCAMP on the pituitary–thyroid system demonstated in this study undoubtedly reflects separate and independent effects on both endocrine glands. Many of the TSH-like effects of cyclic AMP

TABLE II. Dibutyryl Cyclic AMP Treatment and Response of the Pituitary–Thyroid System in the Postnatal Period.

Age and treatment (no. of rats)	Body wt (g ± SE)	Gland wt (mg/100 g body ± SE)		Thyroid ^{125}I (%/mg ± SE)	TSH assay (95% conf. limits)	
		Adrenal	Thyroid		Plasma (mU/100 ml)	Pituitary (mU/mg)
20–24 hr						
Normal (29)	6.3 ± 0.1	39.4 ± 1.4	12.8 ± 0.5	5.5 ± 0.7	<40	2.4 (2.0–2.9)
dbCAMP (29)[a]	6.5 ± 0.1	37.6 ± 1.3	11.7 ± 0.6	12.0 ± 1.5[c]	92 (74–115)[c]	4.1 (3.4–5.1)[c]
72–96 hr						
Normal (42)	7.9 ± 0.4	37.5 ± 1.3	14.8 ± 0.6	1.7 ± 0.6	65 (52– 80)	3.3 (2.5–4.5)
96–120 hr						
dbCAMP (22)[b]	10.3 ± 0.2	33.2 ± 1.0	13.1 ± 0.5	3.1 ± 0.1[c]	111 (90–138)[c]	6.4 (5.3–7.8)[c]
6–7 days						
Normal (38)	17.5 ± 1.1	23.2 ± 1.0	13.3 ± 0.7	0.8 ± 0.1	60 (48– 75)	3.8 (3.2–4.5)
dbCAMP (23)[b]	14.0 ± 0.3	25.0 ± 1.0	12.9 ± 0.8	1.3 ± 0.1[c]	91 (74–119)[c]	7.0 (5.8–8.4)[c]

[a] Newborn of mothers receiving 10 mg of dbCAMP, twice daily, over last 5 days of gestation.

[b] Pups from normal mothers; injected with 0.5 dbCAMP daily, beginning on first postnatal day. Normal controls given saline.

[c] Means differ significantly ($p = 0.05$ or less) from normal value at appropriate time interval.

or its derivatives on the thyroid can be produced *in vitro*. Dibutyryl cyclic AMP stimulates colloid droplet formation, glucose oxidation and ^{32}P incorporation in thyroid slices (10–12). Cyclic AMP administration to T_4 pretreated mice promotes increase in biosynthesis of thyroid protein; enlargement of the gland and histologic activation also occur although endogenous TSH secretion is blocked (25). A direct influence of nucleotides on pituitary TSH secretion, modifiable by the absence or presence of thyroid hormone has been demonstrated. Augmented pituitary cyclic AMP levels and enhanced TSH secretion, resulting from thyroidectomy are restored to normal by a single injection of triiodothyronine (15) and thyroxine completely inhibits the augmentation of TSH secretion *in vitro* induced by dbCAMP or theophylline (16). The paradoxical radiometric changes occurring in the thyroid gland despite the rather uniform elevation of plasma and pituitary TSH levels strongly suggests that in this study dbCAMP administration induced separate and independent effects on both thyroid and adenohypophysis.

Whether or not TRH release from the hypothalamus was also effected in these experiments cannot be stated. The adenyl cyclase–cyclic AMP–phosphodiesterase system of the pituitary thyrotroph apparently is responsive to TRH. The synthetic hypothalamic hormone promotes rapid increase in cyclic AMP levels in the pituitary; the rise precedes the release of TSH (15). Crude hypothalamic extracts are also effective in elevating pituitary cyclic AMP and stimulating TSH release (17). Synthetic TRH, administered to pregnant rats on the last day of gestation also stimulates TSH release from the fetal hypophysis (26). A stimulatory effect of TRH on the *in vitro* release of TSH was also demonstrated for the postnatal pituitary (20). It would be of interest to determine whether the enhanced TSH secretion induced by nucleotide treatment in the present study was a consequence of increased intracellular cyclic AMP levels in the fetal hypophysis.

Summary and Conclusions. Administration of the dibutyryl derivative of 3′,5′-cyclic adenosine monophosphate to virgin and pregnant rats induced significant change in the activity of the pituitary–thyroid system without major alteration in pituitary–adrenocortical function. Nucleotide treatment consistently resulted in elevation of TSH levels in plasma and pituitary. Thyroidal radioiodine uptake was decreased in nonpregnant rats, unaffected in mother and offspring and augmented during the first postnatal week of dbCAMP treatment. It is postulated that dbCAMP exerts (a) separate and independent *in vivo* effects on thyroid and adenohypophysis and (b) enhances TSH, but not ACTH, secretion from the fetal pituitary.

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