Effect of Caffeine on Thyroid and Pituitary Function in Newborn Rats⁽⁴⁰⁾

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Summary

The possibility that caffeine, a central nervous system stimulant used in neonatal apnea, may produce acute or chronic changes in growth hormone (GH), thyroxine (T₄) and thyrotropin (TSH) was studied in the newborn rat. Five-day-old rats were separated into three groups: control (0) group receiving saline, Group I (low dose caffeine) receiving 5 mg/kg and Group II (high dose caffeine) receiving 50 mg/kg. Acute effects were studied at 2, 4, and 24 h after injection. Chronic effects were studied 24 h after the last of 10 daily injections. GH, T₄, and TSH were measured by radioimmunoassay and caffeine by high pressure liquid chromatograph. GH was increased at all times and all doses after a single injection of caffeine. After chronic therapy, the increase in GH was small, suggesting depletion of pituitary reserve. A high dose of caffeine had a biphasic effect on T₄ with an increase at 4 h and a decrease at 24 h. Thyrotropin-releasing hormone (TRH)-induced TSH release at 24 h was not influenced by caffeine administration. Chronic caffeine therapy stimulated both T₄ and TSH; however, TRH-stimulated TSH release was decreased, suggesting that chronic therapy may blunt pituitary TSH response.

Abbreviations

GH, growth hormone
PDE, phosphodiesterase
T₃, triiodothyronine
T₄, thyroxine
TRH, thyrotropin releasing hormone
TSH, thyrotropin

Besides the stimulant effect of the methylxanthines on the central nervous system and on cardiac inotropy and chronotropy, these compounds exert a variety of hormonal and metabolic effects. The administration of two methylxanthines, caffeine and theophylline, to adult rats results in decreased GH, TSH, T₃ and thyroxine T₄ serum levels (29). But in *in vitro* isolated organ preparations, caffeine increases T₄ (3) and TSH (28) release, and theophylline increases the release of GH (32). Published data on the hormonal effects of methylxanthines in humans are contradictory. Peracchi *et al.* (25) and Ensinnck *et al.* (17) observed a decrease in GH level in acromegalic patients after aminophylline. In normal men, Blackard *et al.* (8) and Peracchi *et al.* (25) did not find any modification in basal GH after aminophylline, whereas, Ensinnck *et al.* (17) described a decrease in GH serum level. After a low dose of oral caffeine given to adult healthy men, neither GH, TSH nor T₃ were modified (30). Because caffeine is currently used in the premature neonate for the treatment of apnea, we evaluated the changes in GH, T₄, and TSH after caffeine in the newborn rat.

MATERIALS AND METHODS

Five-day-old male Sprague-Dawley rats were obtained from the Canadian Breeding Farm, St. Constant, Quebec. Two sets of

experiments were respectively designed to evaluate the acute and the chronic effects of caffeine. Each set used three groups: a control group, which received saline; a low dose caffeine group, which received 5 mg/kg of caffeine dissolved in saline; and a high dose caffeine group, which received 50 mg/kg. Caffeine (37) or saline was injected intraperitoneally in a volume of 0.05 ml/rat. Each group consisted of 30-35 rats. In the acute experiment, the animals were sacrificed at various time intervals after a single injection of saline or caffeine: 2, 4, and 24 h. In the chronic experiment, animals were injected daily for a 10 day period. They were sacrificed 24 h after the last injection. The rat pups were kept with their suckling mothers throughout the experiments and maintained on a 12 h light-dark cycle.

At the end of the 24 h experiment, several rats ($n = 6$) of the control group and of the low dose caffeine group received TRH, in order to evaluate the effect of caffeine on TRH-induced TSH release. In the chronic experiment, each of the three groups was divided into two: for the measurement of either basal TSH ($n = 20$) or TRH-stimulated TSH ($n = 10$). Rats receiving TRH (38) were injected intraperitoneally with 100 ng TRH/100 g body weight, 15 min before sacrifice, as described by Walker *et al.* (34). TRH was dissolved in saline. The volume of TRH in saline was 0.05 ml/10 g of body weight given intraperitoneally.

At the termination of all studies, animals were sacrificed by rapid decapitation at the same time of the day, between 14:00 and 14:30. Blood was collected, and plasma or serum obtained was stored at -20°C until subsequent analysis. T₄ and GH were measured in plasma, TSH in serum. T₄ was measured using an Amerlex T₄ radioimmunoassay kit (Amersham Corporation), TSH (34) and GH (33) by double antibody radioimmunoassay with materials supplied by the NIADDK (39). The TSH intraassay variation is 6-8%; interassay variation is 12% and the sensitivity was between 10-15 ng per tube. For the GH assay, the intra- and interassay coefficients of variation determined for a sample mean of 25 ng/ml were 8.9 and 7.2, respectively. The minimum detectable concentration in the radioimmunoassay defined as the concentration of rat GH, which resulted in a binding of [^{125}I] or GH to antiserum that was 2 standard deviations below the mean binding achieved in the absence of GH, was 3.1 ng/ml.

Caffeine and one of its potential metabolites, theophylline, were measured by high pressure liquid chromatography (2). The sensitivity of the caffeine assay was 0.1 mg/liter and the coefficient of variance at 10 mg/liter was 4.0%.

Statistical analysis was performed using two-way analysis of variance (36) or unpaired Student's *t* test. All values are reported as mean $\pm$ S.E.

RESULTS

GH. Figure 1 shows the effects of caffeine on plasma GH levels in the short term experiment. There was a trend to an increase in plasma GH levels in response to both doses of caffeine at all time points examined. The effect increased significantly with the dose. After chronic treatment (Fig. 2) a significant increase ($P < 0.05$)

Fig. 1. Effect of a single administration of saline ($n = 10$), caffeine 5 mg/kg ($n = 10$), and caffeine 50 mg/kg ($n = 9$) on growth hormone (GH) plasma levels (mean $\pm$ S.E.) at different times after injection. * $P < 0.05$ and *** $P < 0.001$, compared to the control group (saline).

Fig. 2. Effect of saline, caffeine 5 mg/kg, and caffeine 50 mg/kg, on growth hormone (GH) plasma levels (mean $\pm$ S.E.) 24 h after a single injection (left) and 24 h after 10 days of treatment (right) ($n = 14$). * $P < 0.05$ and *** $P < 0.001$, compared to the control group (saline).

in plasma GH levels was noted only after a low dose of caffeine (5 mg/kg); thus, the effect of chronic treatment was attenuated when compared to the effect of a single injection of caffeine.

Pituitary-thyroid axis. Figure 3 illustrates the acute effects of caffeine on plasma T_4 . No significant change was noted at 2 h. At 4 h, a high dose of caffeine significantly increased the level of plasma T_4 (3.79 ± 0.13 versus 3.28 ± 0.15 $\mu\text{g/dl}$, $P < 0.05$). In contrast, at 24 h, a high dose decreased significantly the level of plasma T_4 (4.05 ± 0.18 versus 4.81 ± 0.19 $\mu\text{g/dl}$, $P < 0.001$). To confirm this unexpected biphasic effect, the 24 h experiment was repeated in 4-day-old rats instead of 5-day-old animals, so that the rats were sacrificed at the same age as the rats from the 2 and 4 h experiments. A decrease in plasma T_4 concentration after 5 mg/kg of caffeine (1.35 ± 0.11 versus 1.97 ± 0.20 $\mu\text{g/dl}$, $P < 0.01$) was noted. To determine whether this decrease in T_4 level was a direct effect of caffeine on the thyroid gland, or was due to an inhibition of TSH secretion, TRH-induced TSH release was meas-

ured 24 h after either saline (control group) or 50 mg/kg of caffeine (treated group) in the 4-day-old rats. No significant change in TRH-stimulated TSH was noted (377.8 ± 32.5 versus 360.7 ± 37 $\mu\text{g}/100$ ml).

Table 1 shows the effect of chronic caffeine treatment on basal and TRH-stimulated T_4 and on basal and TRH-stimulated TSH. Both low and high doses of caffeine significantly ($P < 0.001$) increased T_4 levels (5.06 ± 0.29 and 6.04 ± 0.22 , respectively, versus 4.12 ± 0.27 $\mu\text{g/dl}$ in 0 dose). The same pattern was found after TRH administration but there was no significant difference in each group between basal T_4 and TRH-stimulated T_4 , suggesting that the 15 min interval between TRH administration and sacrifice was probably too short to produce changes in T_4 concentration. Chronic therapy with caffeine resulted in T_4 levels greater than control animals (Table 1), suggesting an escape phenomenon from the biphasic effect obtained with acute caffeine administration.

Basal TSH was slightly increased by a low dose (17.9 ± 2.57 versus 10.6 ± 1.43 $\mu\text{g/dl}$) and significantly by a high dose of caffeine (25.1 ± 6.93 versus 10.6 ± 1.43 $\mu\text{g/dl}$, $P < 0.05$) (Table 1). The TSH levels after TRH stimulation was considerably greater than basal levels (216.6 ± 45.5 versus 10.6 ± 1.4 $\mu\text{g}/100$ ml); however, chronic caffeine therapy with both low (5 mg/kg) and high doses (50 mg/kg) attenuated the TSH response to TRH stimulation. In the caffeine-treated rats, the TSH levels in the TRH-stimulated rats were much higher than the basal TSH. But TSH levels after TRH stimulation in the low and high caffeine-treated rats were significantly lower compared to the non-caffeine treated group (Table 1), suggesting that chronic caffeine therapy may possibly lead to exhaustion of pituitary reserve.

Caffeine assay. The plasma levels of caffeine and of theophylline obtained after injection of caffeine are shown in Table 2. The peak plasma concentration of caffeine after 5 mg/kg is 5.26 ± 0.38 mg/liter, which is within the lower therapeutic range for the management of neonatal apnea. After 50 mg/kg, the peak concentration is 67.57 ± 2.84 mg/liter, which is supra-therapeutic concentration.

The caffeine level 24 h after the last injection of a chronic treatment with 50 mg/kg (2.00 ± 1.26 mg/liter) is much lower than 24 h after a single injection of 50 mg/kg (20.65 ± 3.36 mg/liter), suggesting that there is no accumulation of the drug and/or that the clearance is greater at 15 days of age than at 5 days. No demethylation of caffeine into theophylline was detected after a single injection of 5 mg/kg.

Fig. 3. Effect of a single administration of saline, caffeine 5 mg/kg, and caffeine 50 mg/kg, on thyroxine (T_4) plasma levels (mean $\pm$ S.E.) at different times after injection. * $P < 0.05$ and *** $P < 0.01$, compared to the control group (saline).

Table I. Effect of chronic treatment by caffeine on basal T_4 , thyrotropin releasing hormone-(TRH)-stimulated T_4 , basal TSH, TRH-stimulated TSH (data are expressed as means $\pm$ S.E.M.)

Caffeine (mg/kg/day) (for 10 days)	T_4 (μ S/100 ml)		TSH (μ g/100 ml)	
	Basal	TRH-stimulated	Basal	TRH-stimulated
0	4.12 $\pm$ 0.27	4.73 $\pm$ 0.44	10.6 $\pm$ 1.4	216.6 $\pm$ 45.5
5	5.06 $\pm$ 0.29 ¹	5.47 $\pm$ 0.32 ²	17.9 $\pm$ 2.6	101.9 $\pm$ 19.5 ³
50	6.04 $\pm$ 0.22 ¹	6.66 $\pm$ 1.53 ²	25.1 $\pm$ 6.9 ³	153.6 $\pm$ 31.7 ³

¹ $P < 0.001$ compared to 0 caffeine dose.² $P < 0.01$ compared to 0 caffeine dose.³ $P < 0.05$ compared to 0 caffeine dose.

DISCUSSION

GH. The wide fluctuations in individual concentrations of plasma GH noted by several investigators (23) may explain the discrepancies we found between the control groups in the acute experiments. In the adult male rat, GH secretion is characterized by an endogenous ultradian rhythm with a period of 3.3 h with synchronization of the pulses between animals (33). In the newborn rat, GH is high (21), and follows an episodic secretion pattern, which is more erratic than that of adult rats (16) with no synchronization of the pulses between animals. Therefore, the time of the sacrifice may have occurred at various points of the GH secretory pattern in individual rats.

At the end of the chronic experiment, *i.e.*, at 15 days of age, the control GH values were lower than in the acute experiments. This decrease parallels the physiologic pattern of plasma GH level in the developing rat. GH level is high in cord blood and during the first days of life, then decreases until 26 days of age (16).

Our study shows a consistent positive effect of caffeine on GH plasma levels in the newborn rat. A blunted response after a chronic treatment relative to a single injection suggests a possible exhaustion of the pituitary gland due to prolonged stimulation by caffeine. The lower plasma caffeine level after 10 days of treatment, compared to 24 h after a single injection, may also be responsible for this smaller effect.

Several mechanisms may explain caffeine stimulation of GH secretion. Methylxanthines are PDE inhibitors, leading to an increase in pituitary cyclic AMP which is involved in GH release (32). The concentrations of caffeine or theophylline required for PDE inhibition are much greater than the therapeutic range. An effect of caffeine on neurotransmitters is also possible. Caffeine has been shown to increase the turnover of norepinephrine (7) and of serotonin (6) in the brain. Both norepinephrine and serotonin stimulate GH secretion (23) in adult rats and humans. Similarly, in the newborn rat, serotonin increases GH release. But the alpha-adrenergic component of GH stimulation is inactive in the neonate (21), suggesting that norepinephrine-mediated GH stimulation is an unlikely mechanism in this age group.

Significant clinical laboratory changes may be expected from these hormonal effects. When used in the newborn infant for the treatment of apnea, caffeine may induce an increase in blood glucose (22) as does theophylline (4, 31), another methylxanthine that can be methylated into caffeine in the newborn (9). It is possible that caffeine-induced increase in GH may play a role in the elevation of blood glucose; however, exogenous GH has been reported to fail to induce major metabolic changes in early infancy (14). Conversely, hyperglycemia has been shown to have a paradoxical positive effect on GH secretion in the newborn infant up to 10 days of life (10), and might participate in the increase in GH after caffeine. The significant increase in GH after chronic administration of a therapeutic dose of caffeine might possibly be of clinical importance. Available data, however, show no difference in growth and development between caffeine-treated premature infants and matched control infants in long term follow-up studies (20).

Pituitary-thyroid axis. Our studies show that an acute single dose of caffeine alters T_4 and TSH. The significant decrease in plasma T_4 noted 24 h after one injection of caffeine is not due to the

comparison with a high control level because this decrease was reproducible when saline or caffeine was given at 4 days of age where the control group had a low T_4 level. This high level in the control group is probably due to increasing age because those rats were sacrificed at 6 days of age. The increase in basal T_4 level between 5 and 6 days is in agreement with the increase found by Walker in the developing rat between 5 and 7 days (35). The decrease in plasma T_4 concentration at 24 h is unlikely to be due to an inhibition of TSH secretion by caffeine because TRH-stimulated TSH is not significantly different with or without caffeine. It may be due either to an increased peripheral metabolism of T_4 into T_3 and/or to a direct inhibition of T_4 secretion by caffeine. The biphasic effect of 50 mg/kg of caffeine on T_4 level is in accord with the *in vitro* experiment of Amiragova (3) on isolated thyroid tissue, wherein caffeine increased T_4 secretion but decreased T_4 synthesis.

Chronic stimulation of T_4 and TSH release is demonstrated after caffeine. The parallelism of the dose-response curves suggests that caffeine increases chronic T_4 secretion by increasing TSH pituitary release. TRH-stimulated TSH release is attenuated by caffeine, suggesting that long-term stimulation by caffeine may blunt the capacity of the pituitary thyrotroph cells to respond to TRH. It may be due to a chronic increase in TSH release with or without inhibition of TSH synthesis after caffeine. This is in accord with the absence of reactivity of TSH to TRH in the few cases of pituitary TSH secreting tumors reported (5). Although our results suggest an effect of caffeine at the pituitary level, a stimulatory effect on the hypothalamus cannot be excluded because we did not measure TRH.

Thyroid hormones play an important role in GH synthesis and secretion in the newborn rat as well as in the adult rat (11). The administration of T_3 results in restoration of the depleted pituitary GH content in neonatal hypothyroid rats, but T_3 induced hyperthyroidism decreases pituitary GH content in previously normal rats. One of the possible explanations would be an "increased fractional removal rate for intrapituitary GH" (11), that would lead to increased plasma GH concentrations. The thyroid stimulation after chronic caffeine therapy might add to the positive effect of caffeine on plasma GH level.

Whether caffeine has the same effects on T_4 and TSH in the newborn infant as in the newborn rat remains to be investigated.

Table 2. Plasma concentration of caffeine and theophylline as a function of dose and age

Age (day)	Time (h)	n	Caffeine (mg/liter)		Theophylline (mg/ liter)	
			5 ¹	50 ¹	5 ¹	50 ¹
5	2	10	3.34 $\pm$ 0.49	67.57 $\pm$ 2.84	ND ²	ND ²
	4	10	5.26 $\pm$ 0.38	59.99 $\pm$ 2.34	ND ²	0.93 $\pm$ 0.11
	24	10	1.52 $\pm$ 0.09	29.65 $\pm$ 3.36	ND ²	1.99 $\pm$ 0.09
15	24	6	ND ²	2.00 $\pm$ 1.26	0.64 $\pm$ 0.07	2.75 $\pm$ 0.58

¹ Dose of caffeine (mg/kg).² ND, Non-detectable. Lower limit of sensitivity = 0.5 mg/liter.

The rat is born with an immature hormonal state and the maturation of the pituitary-thyroid axis proceeds during the first 2 wk of postnatal life (13, 15). In contrast, this maturational process begins at mid-gestation in the human fetus. Thyroid responsiveness to TSH and TSH responsiveness to exogenous TRH are high at birth in the human neonate (18) relative to the rat (27). In the preterm infant, T_3 and T_4 are lower than in the full term infant throughout the first weeks of life (1, 12). Even lower T_3 and T_4 levels have been found in premature babies with respiratory distress syndrome (1, 26). Increasing a previously low T_4 plasma level might be one of the mechanisms by which caffeine or theophylline increase the metabolic rate in the newborn (19, 24).

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