Iodothyronine Release from the Perfused Canine Thyroid following Cessation of Stimulation: *RAPID DECLINE OF TRIIODOTHYRONINES IN COMPARISON WITH THYROXINE*

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The persistently high secretion of iodothyronines despite cessation of TSH infusion is most likely the result of a continued stimulation by receptor-bound TSH. Because the clearance of intracellular cAMP is rapid and the concentration of cAMP used for stimulation in these experiments only exceeded the concentration necessary for eliciting a secretory response modestly, it is reasonable to assume that stimulation of colloid droplet formation stopped shortly after termination of cAMP infusion. The bulk of iodothyronines secreted thereafter thus originated from continued hydrolysis of thyroglobulin engulfed by the follicular cells during the preceding cAMP infusion. […]

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ABSTRACT The kinetics of thyroid secretion after termination of stimulation by 100 μU/ml bovine thyroid stimulating hormone (TSH) or 5 mM cyclic AMP (cAMP) were studied using perfused canine thyroid lobes. All experiments were performed as paired comparisons, one thyroid lobe acting as a control continuing to receive infusion of the stimulator. 2.5 h after termination of TSH infusion, the secretion of thyroxine (T_4), 3,5,3′-triiodothyronine (T_3), and 3,3′,5′-triiodothyronine (rT_3) was not significantly different from that of the control lobes. After cessation of cAMP infusion, the secretion of T_4 continued unaffected for ~40 min. Then a gradual decline in T_4 release occurred. The secretion of T_3 and rT_3 decreased somewhat earlier leading to a transient phase with increases in the T_4:T_3 and T_4:rT_3 ratios in the thyroid effluent.

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INTRODUCTION

The use of specific and sensitive radioimmunoassays for quantitation of iodothyronine release from perfused thyroid lobes has revealed that iodothyronines are secreted from the thyroid in mutual proportions quite different from those found in hydrolysate of thyroid tissue, and that stimulation of thyroid secretion induces alterations in the relative proportions of various iodothyronines in secretion (1–3). In view of the different biological activities of thyroxine (T_4)[1] and 3,5,3′-triiodothyronine (T_3), it is important to elucidate in detail the mechanisms whereby the composition of thyroid secretion is altered and to learn the secretory pattern in various functional states of the thyroid gland.

In the present study we measured T_4, T_3, and 3,3′,5′-triiodothyronine (reverse T_3, rT_3) in thyroid effluent after cessation of stimulation with thyroid stimulating hormone (TSH) or cyclic AMP (cAMP). The method used for the experiments, as in other studies presented earlier, is a once-through perfusion of isolated thyroid lobes with a hormone-free medium. This technique permits more accurate and dependable estimations of the kinetics of thyroid secretion than in vivo studies, which are hampered by the problems of recirculation of stimulators and previously secreted iodothyronines. Furthermore, the delays in diffusion inherent in studies of thyroid slices or incubated lobes are also avoided in the present model

[1] *Abbreviations used in this paper*: cAMP, cyclic AMP; rT_3, 3,3′,5′-triiodothyronine; T_4, thyroxine; TSH, thyroid-stimulating hormone; T_3, 3,3′,5′-triiodothyronine.

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because stimulators and secretory products are transported via the circulation.

METHODS

Two-sided thyroid perfusions were performed in 10 mongrel dogs weighing 20–28 kg. Individual data on weight and iodothyronine content of thyroid lobes are given in Tables I and II. The technique has been described in detail earlier (1, 2). In brief, the two separate thyroid lobes are isolated *in situ* in such a way that perfusion medium pumped into an isolated segment of each common carotid artery can be collected quantitatively from each thyroid lobe through catheters in the internal jugular veins. The dog is exsanguinated after the insertion of the afferent catheters. The perfusion medium is a modified Krebs-Ringer bicarbonate buffer containing 4% dextran (70,000 mol wt). The flow rate for each thyroid lobe is 0.63 ml/min. The perfusion pressure was constant throughout the experiments at 30–40 mm Hg. The total effluent from 5-min intervals was collected. In all experiments 100 μU/ml TSH or 5 mM cAMP was infused in both thyroid lobes as soon as both of the afferent catheters had been inserted in the common carotid arteries, i.e., TSH or cAMP were infused for ~20 min while the dog was exsanguinated and the efferent catheters were inserted in the internal jugular veins. As soon as the first effluent sample for determination of iodothyronines had been obtained after 30 min of perfusion in the final setup (the equilibration period generally employed in this model [1]), one thyroid lobe was switched to control medium perfusion for the rest of the perfusion period while the other continued to receive TSH or cAMP. Shift to control medium was performed alternately in the left and right thyroid lobe. After 200 min of perfusion both thyroid lobes were removed and hydrolysed with pronase using the method of Inoue and Taurog (4) as earlier employed (3).

TABLE I

T_4, T_3, and rT_3 in Thyroid Effluent and Hydrolysate following TSH Stimulation

Experiment		Weight of lobe	T_4			T_3			rT_3		
			Effluent		Hydrolysate	Effluent		Hydrolysate	Effluent		Hydrolysate
		mg	ng/ml		ng/mg	ng/ml		ng/mg	ng/ml		ng/mg
			25–30*	175–200‡		25–30	175–200		25–30	175–200	
1	A§	790	18.2	89 ±4	812	4.32	12.6 ±1.0	73.4	1.04	2.92 ±0.02	13.0
	B‖	637	23.8	86 ±3	869	5.22	12.5 ±0.9	75.0	1.38	2.07 ±0.01	13.2
2	A	796	24.3	118 ±4	884	4.54	17.6 ±0.9	75.0	0.92	3.23 ±0.04	12.5
	B	987	31.2	121 ±6	934	4.87	20.7 ±0.6	77.0	1.07	3.45 ±0.04	12.2
3	A	704	43.1	208 ±8	1,354	8.22	23.7 ±0.4	70.2	1.84	4.30 ±0.24	14.7
	B	555	18.4	132 ±11	1,391	4.72	15.6 ±1.3	72.6	1.01	3.33 ±0.20	14.9
4	A	701	25.9	156 ±4	968	11.68	41.7 ±1.9	120.0	1.09	3.54 ±0.25	9.7
	B	925	39.3	128 ±3	946	10.68	35.2 ±1.4	116.9	1.82	3.17 ±0.05	10.2
Mean ±SD	A	748 ±26	27.9 ±5.3	143 ±26	1,004 ±120	7.19 ±1.74	23.9 ±6.4	84.6 ±11.8	1.22 ±0.21	3.50 ±0.30	12.5 ±1.0
	B	776 ±106	28.2 ±4.5	117 ±10	1,035 ±120	6.37 ±1.44	21.0 ±5.0	85.4 ±10.5	1.32 ±0.19	3.01 ±0.32	12.6 ±1.0
P¶		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

* Sample obtained during the 25–30-min interval of perfusion.

‡ Mean±SD of three samples obtained during the 175–200-min interval of perfusion.

§ Lobe receiving 100 μU/ml TSH throughout the experimental period.

‖ Lobe receiving 100 μU/ml TSH until 30 min of perfusion.

¶ NS = $P > 0.05$ (paired t test).

TABLE II

T_4, T_3, and rT_3 in Thyroid Effluent and Hydrolysate following cAMP Stimulation

Experiment		Weight of lobe	T_4				T_3				rT_3			
			Effluent			Hydrolysate	Effluent			Hydrolysate	Effluent			Hydrolysate
		mg	*ng/ml*			*ng/mg*	*ng/ml*			*ng/mg*	*ng/ml*			*ng/mg*
			25–30*	65–90‡	175–200‡		25–30	65–90	175–200		25–30	65–90	175–200	
1	A§	965	16.8	78.4 ±12.6	55.1 ±3.5	658	3.96	19.1 ±2.1	14.7 ±0.7	83.1	1.31	3.73 ±0.16	2.35 ±0.29	14.1
	B‖	1,148	20.0	70.1 ±1.2	11.3 ±0.4	639	6.93	11.7 ±0.6	4.4 ±0.4	73.0	1.10	3.02 ±0.45	0.70 ±0.08	12.7
2	A	722	5.0	26.1 ±3.4	24.1 ±2.7	1,030	1.27	4.0 ±0.3	4.5 ±0.1	83.9	0.46	0.94 ±0.09	0.94 ±0.11	16.9
	B	767	12.2	43.1 ±2.9	3.2 ±0.2	1,076	3.33	5.0 ±1.1	0.9 ±0.1	83.5	0.71	1.37 ±0.27	0.19 ±0.02	19.1
3	A	726	10.7	74.4 ±21.8	68.9 ±4.1	658	2.76	11.1 ±1.1	12.4 ±0.5	63.3	0.90	2.85 ±0.17	2.38 ±0.11	12.3
	B	776	10.4	64.9 ±2.3	12.6 ±2.5	884	2.40	9.1 ±0.2	2.7 ±0.2	73.4	0.82	1.85 ±0.10	0.66 ±0.05	13.3
4	A	711	8.4	100.0 ±9.3	67.6 ±12.4	714	5.04	22.6 ±2.5	21.9 ±2.3	84.7	0.70	3.31 ±0.08	2.48 ±0.42	11.4
	B	653	11.7	114.9 ±8.1	7.3 ±1.5	696	6.10	19.4 ±0.6	4.5 ±0.7	80.7	0.92	3.62 ±0.27	0.34 ±0.03	10.4
5	A	950	1.7	24.3 ±8.8	51.1 ±5.2	1,034	0.60	8.8 ±2.0	12.6 ±0.7	111.2	0.13	1.08 ±0.26	1.29 ±0.08	16.9
	B	850	1.9	29.0 ±1.8	4.7 ±0.5	978	0.70	3.3 ±0.3	1.3 ±0.1	112.8	0.17	0.74 ±0.03	0.19 ±0.01	14.5
6	A	644	10.8	52.0 ±1.9	72.2 ±3.2	382	2.92	10.4 ±0.4	15.7 ±1.7	40.3	0.54	1.36 ±0.19	1.49 ±0.09	3.8
	B	520	8.7	32.5 ±5.2	2.7 ±0.1	291	2.88	4.0 ±0.4	0.9 ±0.2	31.0	0.52	0.64 ±0.09	0.09 ±0.01	2.6
Mean ±SD	A	786 ±56	8.1 ±2.1	59.2 ±12.4	56.5 ±7.3	746 ±102	2.76 ±0.67	12.7 ±2.8	13.6 ±2.3	77.8 ±9.7	0.67 ±0.16	2.21 ±0.50	1.82 ±0.27	12.6 ±2.0
	B	786 ±86	10.8 ±2.4	59.1 ±13.1	7.0 ±1.7	761 ±116	3.72 ±0.96	8.8 ±2.5	2.4 ±0.7	75.7 ±10.8	0.71 ±0.13	1.87 ±0.50	0.36 ±0.11	12.1 ±2.2
P¶		NS	NS	NS	<0.001	NS	NS	<0.05	<0.01	NS	NS	NS	<0.001	NS

* Sample obtained during the 25–30-min interval of perfusion.

‡ Mean ± SD of three samples obtained during the 65–90- or 175–200-min interval of perfusion.

§ Lobe receiving cAMP, 5 mM throughout the experimental period.

‖ Lobe receiving cAMP, 5 mM until 30 min of perfusion.

¶ *P* value, NS = $P > 0.05$ (paired *t* test).

T_4, T_3, and rT_3 in effluent samples and ethanol extracts of hydrolysates were measured radioimmunologically (5, 6, 3). All samples from one experiment were measured in triplicate in the same assay. Bovine TSH for stimulation was the international standard preparation, a gift from the Medical Research Council, London, England. cAMP was obtained from Sigma Chemical Co., St. Louis, Mo.

Calculation of a hypothetical T_4:T_3 ratio in thyroid effluent. For the elucidation of the results obtained in the experiments presented here, it was interesting to calculate a hypothetical variation in the T_4:T_3 ratio in thyroid effluent using data from four previous thyroid perfusion experiments (3). In these experiments iodothyronines in effluent were measured at short intervals (every 10 min) throughout the experiments. Four control samples were obtained (after 30, 40, 50, and 60 min of perfusion), then 100 μU/ml TSH was infused for 140 min. T_4 and T_3 were also measured in pronase hydrolysate of the thyroids. For calculations the following assumptions were made: the T_4:T_3 ratio in thyroglobulin engulfed and hydrolysed during secretion was identical to that measured in pronase hydrolysate of thyroid homogenate; a constant fraction of T_4 liberated during intracellular hydrolysis of thyroglobulin was deiodinated to T_3 during secretion; T_4 was released somewhat slower than T_3 during hydrolysis of thyroglobulin. In the chosen example the delivery of 75% of the T_4 was estimated to be delayed 20 min in comparison with the T_3.

During steady-state secretion, this delay in T_4 release would not affect the T_4:T_3 ratio in thyroid effluent, and differences between the T_4:T_3 ratio in pronase hydrolysate of thyroid

tissue and in thyroid effluent would be solely the result of T_4 monodeiodination to T_3. The reason for applying the calculations to the above-mentioned experiments was that the stable period of control perfusion before stimulation was necessary for calculation of T_4 monodeiodination to T_3. The difference in the molecular weights of T_4 and T_3 was corrected when necessary.

In the following equation:

$$\text{fraction } T_4 \rightarrow T_3 = \left(\text{control } T_{3\,\text{eff}} - \frac{\text{control } T_{4\,\text{eff}}}{T_4/T_3 \text{ in thyr}}\right) \Big/ \text{control } T_{4\,\text{eff}}, \quad (1)$$

where fraction $T_4 \rightarrow T_3$ is the fraction of liberated T_4 deiodinated to T_3 during secretion (assumption 2). Control $T_{3\,\text{eff}}$ and control $T_{4\,\text{eff}}$ are the mean T_3 and T_4 concentrations in thyroid effluent during control perfusion. T_4:T_3 in thyr is the T_4:T_3 ratio in pronase hydrolysate of thyroid homogenate.

$$T_3(TG) = T_{3\,\text{eff}} - (\text{fraction } T_4 \rightarrow T_3 \times T_{4\,\text{eff}}) \quad (2)$$

where T_3(TG) is the calculated amount of T_3 in an effluent sample originating from T_3 in thyroglobulin. $T_{3\,\text{eff}}$ and $T_{4\,\text{eff}}$ are the measured concentrations of T_3 and T_4 in the same effluent sample. T_3(TG) was calculated for each effluent sample.

$$T_4 \text{ hypot} = T_4{:}T_3 \text{ in thyr} \times (0.75 \times T_3[TG]_{[-20\,\text{min}]} + 0.25 \times T_3[TG]) \quad (3)$$

where T_4 hypot is the hypothetical T_4 concentration in each sample under the assumption that the delivery of 75% of T_4 is delayed 20 min to T_3 during secretion. $T_3(TG)_{(-20\,\text{min})}$ is T_3(TG) in the effluent sample obtained 20 min earlier. T_4 hypot was calculated for each sample. The hypothetical T_4/measured T_3 was then calculated for each sample and the curve compared with the curve drawn from the raw experimental data.

Because the fractional deiodination of T_4 to rT_3 seems to diminish during TSH stimulation (3), such calculations could not be made for rT_3. As to the amounts of T_4 disappearing during secretion as a result of deiodination to T_3 and rT_3, these cannot be estimated exactly and are not corrected. Judged from the variation in T_4 secretion during inhibition of intrathyroidal deiodination (7), this amount of T_4 is relatively small compared with the T_4 secreted. In the four experiments used for the present calculations, fraction $T_4 \rightarrow T_3$ was calculated to be 4.3, 5.0, 8.3, and 11.0%, respectively. Furthermore, a small fraction of T_3 also seems to be deiodinated during secretion[2] that would tend to correct the small error.

Student's *t* test for paired comparisons was applied for statistical analyses, employing a 5% limit of significance. For the comparison of ratios the test was applied to the reciprocal ratios: T_3:T_4 and rT_3:T_4, to avoid the markedly abnormal distribution characteristic of ratios larger than one.

RESULTS

Thyroid secretion after cessation of TSH infusion. The mean T_4 concentrations in effluent from four thyroid lobes that received a constant infusion of 100 μU/ml TSH from the beginning of the perfusion and during the full experimental period, and the four contralateral thyroid lobes that received control medium after 0.5 h are depicted in Fig. 1. The T_4 secretion was already increasing rapidly when the first sample for iodothyronine measurements was obtained. The increase was nearly linear for ~1 h, then the curves leveled off, and a slight, gradual decrease in T_4 release was observed. The shape of the two curves was nearly identical.

Individual data for T_4, T_3, and rT_3 in the first sample and in samples obtained at the end of the experiments are given in Table I. There was no significant difference in iodothyronine release either at the start or at the end of the experiment. Thus the cessation of TSH infusion 2.5 h earlier had not resulted in a decrease in thyroid iodothyronine release.

To investigate whether the cessation of TSH infusion induced alterations in the mutual proportions between various iodothyronines in thyroid effluent, the T_4:T_3 and T_4:rT_3 ratios were calculated for all samples. Both the T_4:T_3 and the T_4:rT_3 ratios in effluent were always considerably lower in thyroid effluent than in thyroid hydrolysate. The variations in ratios in effluent were similar to those observed during continuous cAMP infusion (Figs. 3 and 5). Cessation of TSH infusion did not induce any discernible alteration in the relative

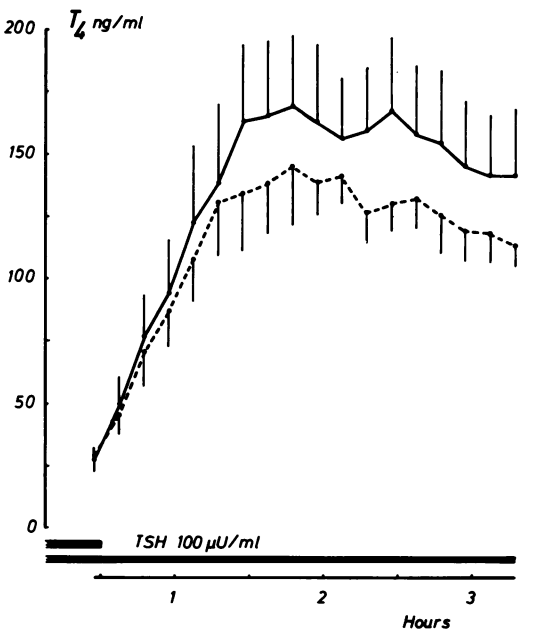

FIGURE 1 T_4 in effluent from dog thyroid lobes during once-through perfusion. In each dog both of the two separate lobes were perfused independently. Both lobes received 100 μU/ml TSH during the final part of the surgical procedure, ~20 min before the beginning of the experimental period. One thyroid lobe (——) received control medium after 30 min, when the first sample for determination of iodothyronines had been obtained, whereas the contralateral lobe (– – –) continued to receive TSH during the experimental period. (Mean±SE, $n = 4$).

[2] Laurberg, P., unpublished observations.

composition of thyroid effluent, the curves from the two lobes being superimposable.

Thyroid secretion after cessation of cAMP infusion. To exclude a continued stimulation by receptor-bound TSH, a similar series of experiments was made using 5 mM cAMP as stimulator of thyroid secretion. The T_4 release in these experiments is shown in Fig. 2. In thyroid lobes receiving cAMP throughout the experimental period, the shape of the T_4 release curve was similar to that observed during TSH infusion. However, cessation of cAMP infusion induced marked alterations in T_4 release, the curve assuming a bell shape. During the first 40 min after cessation of cAMP infusion, the T_4 release continued to increase, very similarly to that from the contralateral thyroid lobes still receiving cAMP. After 40 min the curve leveled off, followed by a rapid decrease in T_4 secretion which then gradually approached a level comparable to that observed from unstimulated thyroid lobes.

Again the T_4:T_3 and T_4:rT_3 ratios in effluent samples were calculated. The T_4:T_3 ratios are depicted in Fig. 3. During continuous infusion of 5 mM cAMP an initial increase in the T_4:T_3 ratio in effluent was observed. This is the late ascending phase of the variation in ratios observed after initiation of stimulation. The full response is depicted in Fig. 4. On the other hand, after cessation of cAMP infusion a much steeper in-

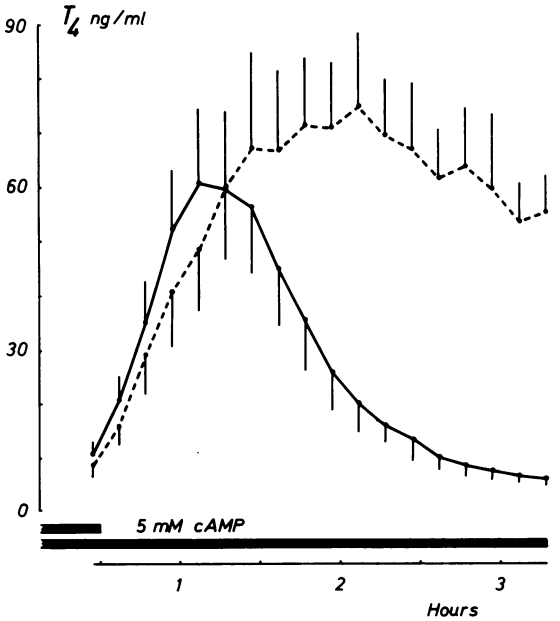

FIGURE 2 T_4 in thyroid effluent in six experiments. The two separate thyroid lobes were perfused independently. Both lobes received 5 mM cAMP during the final part of the surgical procedure and during the first 30 min of the experimental period. After 30 min one lobe (——) was perfused with control medium, whereas the other (– – –) continued to receive cAMP for the entire experimental period. (Mean±SE).

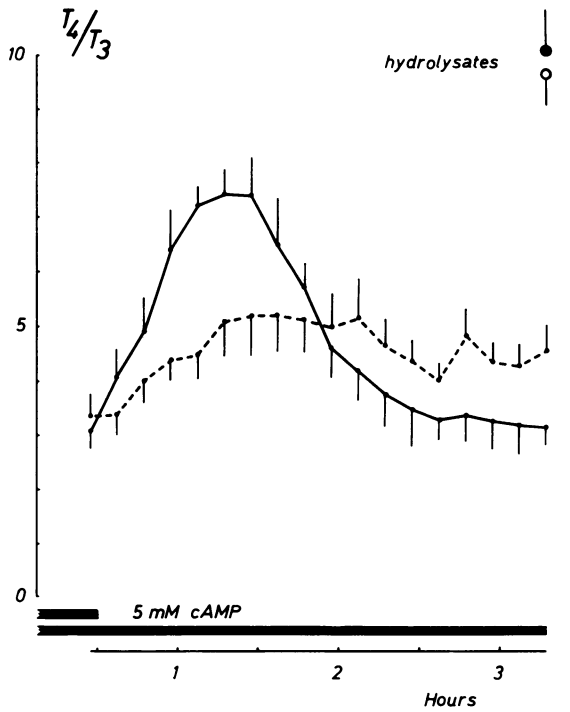

FIGURE 3 T_4:T_3 ratios (wt/wt) in thyroid effluents and thyroid hydrolysates in the six experiments shown in Fig. 2. ——, effluent of thyroid lobes where cAMP infusion was stopped after 30 min; ●, hydrolysate of these lobes. – – –, effluent of thyroid lobes continuing to receive cAMP; ●, hydrolysate of these lobes. (Mean±SE).

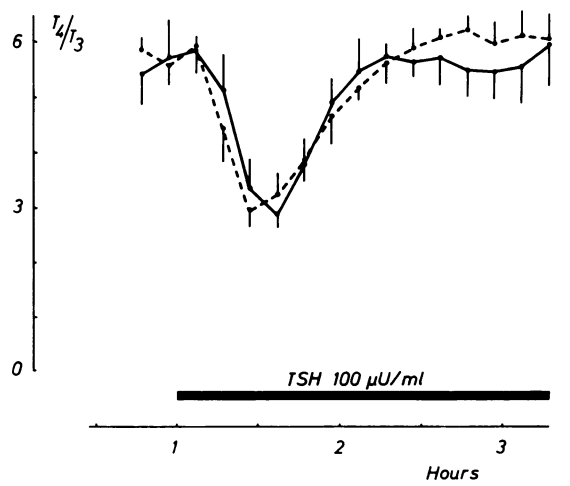

FIGURE 4 T_4:T_3 ratio (wt/wt) in thyroid effluent in four experiments where infusion of 100 μU/ml TSH was initiated after 1 h. – – –, ratio calculated directly from the measured T_4 and T_3 in effluent. ——, ratio calculated from the measured T_3 in effluent and from a calculated T_4 secretion. The most important assumptions made for calculating this hypothetical T_4 secretion were that a constant fraction of T_4 is monodeiodinated to T_3 during secretion, and 75% of T_4 is postponed 20 min relative to T_3 during release from intracellular thyroglobulin.

crease in the T_4:T_3 ratio was seen, and at the time that T_4 release was maximal, and similar to that from the continuously stimulated lobes (Table II, 65–90 min of perfusion), the T_4:T_3 ratio in thyroid effluent was much higher than that in effluent from the contralateral lobes. As it appears from Table II this reflects that the T_3 release leveled off earlier than the T_4 release after withdrawal of cAMP and that the T_3 release from these lobes—when their secretion was maximal—was lower than from the continuously stimulated lobes. During the period with decreasing T_4 secretion, the T_4:T_3 ratio in effluent also decreased and at the end of the experiment it was lower in the unstimulated than in the stimulated thyroid lobes ($P < 0.01$). The T_4:T_3 ratio in thyroid effluent was consistently lower than that of thyroid hydrolysate.

As shown in Fig. 5 the T_4:rT_3 ratio in effluent was also significantly enhanced by the cessation of cAMP infusion ($P < 0.05$ in the 65–90-min period of perfusion). However, the effect was less pronounced than on the T_4:T_3 ratio, and even if rT_3 release was lower after withdrawal of cAMP infusion in the 65–90-min interval than during continuous cAMP infusion, this was not statistically significant (Table II). This reflects that the coincidental variation in the relative composition of effluent from the two perfused thyroid lobes in a dog is less than the coincidental variation in absolute release of iodothyronines. When thyroid secretion declined, a fall in the T_4:rT_3 ratio occurred. At the end of the experiment it was statistically significantly lower in effluent from unstimulated than from cAMP-stimulated thyroid lobes ($P < 0.01$).

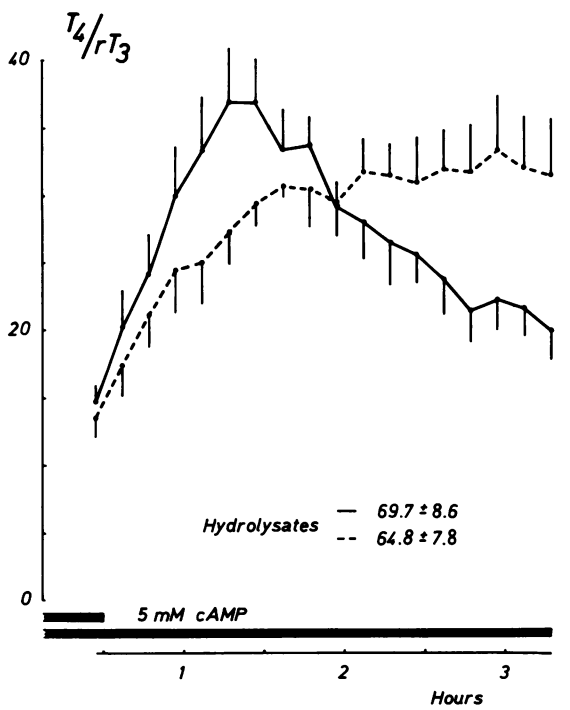

FIGURE 5 T_4:rT_3 ratios (wt/wt) in thyroid effluents and hydrolysates in the six experiments shown in Fig. 2. ——, effluent of thyroid lobes where cAMP infusion was stopped after 30 min. – – –, effluent of thyroid lobes continuing to receive cAMP. (Mean±SE).

Hypothetical T_4:T_3 variation assuming a delay in T_4 release. It was natural to search for a common mechanism for the transient increase in the T_4:T_3 and T_4:rT_3 ratios in thyroid effluent after termination of stimulation, observed in the present study, and the transient decrease in the same ratios in effluent after initiation of stimulation, observed in previous studies (1–3, 8). One possible mechanism was that T_3 and rT_3 in intracellular thyroglobulin in some way is released faster than T_4. To see whether such a mechanism may fit the experimental data obtained using the perfused canine thyroid, and to get an idea of the delay in T_4 release necessary for inducing the observed variation, we took the liberty of including some calculations of a hypothetical variation in effluent T_4:T_3 using data from a previous study where a control perfusion period was followed by a TSH stimulation (3). Hypothetical curves for T_4:T_3 in thyroid effluent were calculated using various delays in various fractions of T_4. Fig. 4 shows the calculated variation in T_4:T_3 in effluent assuming that 25% of T_4 and all T_3 in thyroglobulin engulfed for hydrolysis was secreted at equal rates although the release of 75% of T_4 was postponed 20 min. Further, the mean T_4:T_3 curve actually observed in the experiments is shown. As can be seen, by using this delay in T_4 secretion for calculation, the hypothetical and the experimental curves are practically identical.

DISCUSSION

No discernible alteration in thyroidal secretion was observed during a period >2.5 h after termination of TSH administration. The prolonged effect of TSH is compatible with a slow release of receptor-bound TSH (9, 10). To circumvent the continued stimulation of colloid uptake by receptor-bound TSH, 5 mM cAMP was used as a stimulator. This dose induced a considerable increase in secretion in our preparation, whereas concentrations of cAMP <1 mM had no effect (8). The half-life of endogenous cAMP in stimulated dog thyroid cells has been calculated to be 1 min and 50 s (11). If exogenous cAMP is metabolized similarly in the present experiments, it would be expected that intracellular cAMP should fall below the stimulatory threshold ~5 min after the termination of cAMP infusion. Indeed, when cAMP infusion was terminated a very different pattern of secretion was observed. T_4 release continued unaffected for ~40 min, then it leveled off and gradually declined.

The secretory patterns seen after withdrawal of TSH

and cAMP infusion are in keeping with previous studies suggesting that stimulation of thyroid secretion continues for some time after cessation of TSH administration as a result of persistent activity of bound, biologically active TSH (12–15); and even after that, increased secretion may continue for a certain period because of hydrolysis of intracellular thyroglobulin (14, 16).

Using the same preparation we previously demonstrated that T_3 and rT_3 are secreted preferentially to T_4, i.e., the T_4:T_3 and T_4:rT_3 ratios are consistently higher in thyroid hydrolysate than in thyroid effluent (1, 3). This difference is at least partially the result of intrathyroidal monodeiodination of T_4 to T_3 and rT_3 (7). In the present study our previous finding of a preferential secretion of T_3 and rT_3 was confirmed. However, the most interesting observation concerning the relative composition of thyroid secretion was that after termination of cAMP infusion the induced augmentation of T_4 release persisted longer than that of T_3 and rT_3, leading to a transient increase in the T_4:T_3 and T_4:rT_3 ratios in the effluent.

Previous studies dealing with the secretory dynamics during the early stimulatory phase have shown exactly the opposite pattern of variation of the T_4:T_3 and T_4:rT_3 ratios i.e., a transient decrease (1–3, 8). Three alternative mechanisms for the phenomenon were envisaged. (*a*) enhanced T_4 monodeiodination to T_3 and rT_3. This is unlikely because the phenomenon is not inhibited by propylthiouracil (17). (*b*) an initial preferential ingestion of thyroglobulin relatively rich in T_3 and rT_3, but this seems hardly probable in view of the opposite variation in T_4:T_3 and T_4:rT_3 observed after termination of stimulation. (*c*) we are therefore left with the third possibility; that T_3 and rT_3 are liberated at a higher rate than T_4 from the follicular cells into the circulation. Such a mechanism would explain why the changes in the T_4:T_3 and T_4:rT_3 ratios only appear when the secretory rate changes.

Using data obtained in a previous study on iodothyronine release from perfused thyroid lobes after initiation of stimulation, we have tried to substantiate this theory using a hypothetical model where the liberation of T_4 was delayed in comparison with T_3. It was found that if 75% of the liberated T_4 was delivered to the circulation 20 min later than the remainder of the T_4 and of T_3, then the hypothetical T_4:T_3 curve was an almost exact replica of the experimental one. It is realized that the thyroxine molecules are hardly treated in such two completely separate entities. It seems more probable that the delays of individual T_4 molecules are distributed within a range, in the experiments used for calculation the secretory dynamics happen to be fairly well described by the two populations.

A faster release of T_3 than of T_4 could be the result of either a faster liberation of T_3 from thyroglobulin during intracellular hydrolysis or to a faster transport (or diffusion) of liberated T_3 out of the follicular cell into the capillary. Unfortunately, very little is known of the transport mechanism. On the other hand, thyroglobulin hydrolysis has been rather intensively studied, and it is known that various iodinated compounds may be released at different rates during enzymatic hydrolysis (18–23). We have found in preliminary experiments using dog thyroid homogenate that at acid pH, which is presumably also present in intracellular phagolysosomes, larger fractions of T_3 and rT_3 than of T_4 are released during partial autolysis (24). Thus, with our present knowledge it seems most likely that a faster liberation of T_3 and rT_3 takes place during intracellular hydrolysis of thyroglobulin.

Several factors are responsible for the amount of various iodothyronines secreted from the thyroid in any given situation. The basal factors seem at present to be the iodothyronine content of thyroglobulin; the rate of thyroglobulin uptake in the cells; intrathyroidal iodothyronine deiodinating processes; and differences in the rate of release of various iodothyronines from intracellular thyroglobulin. Other factors such as variations in the activity of hydrolysing enzymes or in the susceptibility of thyroglobulin molecules to hydrolysis could also be involved.

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