Intrathecal Triiodothyronine Administration Causes Greater Heart Rate Stimulation in Hypothyroid Rats than Intravenously Delivered Hormone

Evidence for a Central Nervous System Site of Thyroid Hormone Action

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Abstract

To determine whether intracerebrally localized iodothyronines produce thyroid hormone-related functional effects, heart rate responses were compared in conscious hypothyroid rats given triiodothyronine (T_3) by either the intrathecal or the intravenous route. A significant increase in heart rate occurred within 18 h after 1.5 nmol T_3/100 g body wt was delivered intrathecally through a cannula previously placed in the lateral cerebral ventricle. Injection of the same T_3 dose intravenously through an indwelling jugular catheter or injection of vehicle only by either route produced no significant increase in heart rate during the 48-h postinjection period of observation. These differences were observed even though integrated serum T_3 concentrations were significantly lower after intrathecal than after intravenous T_3 injection. The results indicate that thyroid hormone effects on heart rate are exerted within the brain as well as within the heart.

Introduction

Recent observations have demonstrated that brain iodothyronines are under strong homeostatic control (1), cross the blood-brain barrier through a high-affinity transport mechanism (2), are actively metabolized (3, 4) and processed in discrete neural pathways (5), are concentrated in synaptosomes (3, 6), and bind with high affinity to a limited number of sites in nerve cell nuclei (7) and synaptosomes (8). Thus far, however, only indirect evidence suggests that intracerebrally localized thyroid hormones produce changes in neural structure (9) or function (10).

To test for a causal relationship between brain iodothyronines and nervous system activity, we measured the effect of a single injection of triiodothyronine (T_3)[1] or vehicle given intrathecally on an adrenergically responsive process, heart rate, in conscious hypothyroid rats. Although the amount of T_3 given (1.5 nmol/100 g body wt) was considerably less than that previously reported to cause an increase in heart rate when given systemically as a single injection (11), intrathecal administration of this dose was followed within 18 h by a significant increase in heart rate. Under similar conditions, no increase in heart rate was observed in rats given thyroxine (T_4) or reverse triiodothyronine (rT_3) intrathecally. Moreover, when heart rate response to intrathecal T_3 was compared with the response after intravenous T_3 or vehicle given by either route, a significant increase in heart rate was noted only in animals given T_3 intrathecally.

Methods

Surgically thyroidectomized rats received from Zivic-Miller Laboratories (Allison Park, PA) were given free access to Purina chow (Ralston Purina Co., St. Louis, MO) and 0.5% $CaCl_2$ as drinking water. All rats showed the expected plateau in body weight gain; the duration of hypothyroidism was 3–6 wk in the first and second experiments and 2–4 wk in the third and fourth experiments. Rats used in any one experiment were of similar age, were thyroidectomized on the same day, and were housed in a laboratory space dedicated exclusively to the experiment. All animal care was administered and all treatments were carried out by a single investigator.

Experiment no. 1. Rats were paired by body weight (range, 200–250 g) and were handled as pairs throughout. Rats were anaesthesized with intraperitoneal nembutal (40 mg/kg) and atropine (0.1 mg/kg), and a 23-gauge cannula was lowered through a burr hole in the left lateral cerebral ventricle (coordinates: 0.6 mm posterior to bregma, 1.5 mm lateral to saggital sinus, 3.0 mm below dura), using stereotactic guidance (12). A small incision was made over the precordial area and a fine teflon-coated Medwire (50 μM; Medwire Corp., Mt. Vernon, NY), bared at its end, was sutured to the subcutaneous fat and passed subcutaneously until exteriorized at the nape of the neck; a second length of Medwire was placed subcutaneously over the cranium to act as a reference electrode. The free ends of both wires were placed in a male microconnector strip (2 × 5 × 10 mm) and secured along with the intraventricular cannula to the skull with self-tapping screws and Craniofacial Cement (Plastic Products, Roanoke, VA). 2 d later, when the rats were fully recovered, the monitoring leads were connected via a female microconnector strip and flexible protected cable to a preamplifier and recorder wired to an electronic timer; this was arranged as to allow the animals to move about freely in their cages. Heart rate was recorded simultaneously every 30 min in each member of the pair. After 18 h of recording to allow adaptation to the equipment and obtain base-line values, one rat of each pair was given 1.5 nmol T_3/100 g body wt in 4 μl of 0.2 N NaOH, which was delivered through the cannula over 120 s, while the other rat of the pair received 4 μl of 0.2 N NaOH. The interval between injections rarely exceeded 15 min; injections were made in the afternoon, generally between 2 and 5 p.m. Recordings were continued for 48 h after intrathecal T_3 or NaOH administration. Data recorded during the 12-h period before injection were averaged to calculate base-line values; the overall level of heart rate response was derived from values averaged over the 48-h postinjection period of observation. Four successive mean counts were averaged to obtain a mean heart rate for each 2-h postinjection period; corresponding values obtained from vehicle-treated (n = 5) or T_3-treated (n = 5) rats were then averaged to obtain an overall mean 2-h value±SD.

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1. *Abbreviations used in this paper:* bpm, beats per minute; df, degrees of freedom; rT_3, reverse triiodothyronine; T_3, triiodothyronine; T_4, thyroxine.

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Experiment no. 2. To control for the possibility of a nonspecific effect of intrathecally administered iodothyronine, rats prepared and monitored as described in the first experiment were given 1.5 nmol/100 g body wt of T_4 (n = 4), rT_3 (n = 5), or NaOH (n = 4) intrathecally.

Experiment no. 3. To compare the heart rate-modifying effect of intravenous T_3 with that of intracerebral T_3, silicone jugular venous catheters (0.037 cm o.d., Silastic; Dow Corning Corp., Midland, MI) were implanted in hypothyroid rats (150–200 g body wt) otherwise prepared and monitored as described in the first experiment. Pairs of rats were given 1.5 nmol T_3/100 g body wt or NaOH by intravenous or intrathecal injection (see experiment 4 for intravenous injection volumes) and were observed simultaneously (n = 6 in each category).

Experiment no. 4. To compare changes in serum T_3 concentrations after intrathecal and intravenous administration, matched pairs of hypothyroid rats averaging 260 g body wt had the required cannulas implanted under anaesthesia; 2 d later, seven rats received 3.9 nmol T_3 in 4.0 μl of 2 N NaOH intrathecally and four rats received the same amount of intravenous T_3 given in 130 μl of 6×10^{-4} NaOH through the indwelling silicone tubing and followed by 1 ml of saline. Experiments with ^{125}I-T_3 revealed no adsorption of T_3 to the tubing. 1 ml of blood for T_3 measurement was withdrawn and replaced by an equal volume of saline immediately before T_3 administration, and again at 5 min (preceded by 0.02 ml blood withdrawn and discarded at that interval only) and the other time intervals shown on the abscissa in Fig. 4. Serum was separated in a refrigerated centrifuge and T_3 was measured by radioimmunoassay (13). All determinations were made in a single assay; the intraassay coefficient of variation was ~5%.

Data analysis. To avoid overestimation of significant differences in analyzing sequential heart rate changes, the method of multivariate analysis of variance for repeated measures was chosen. This method allows correlations between time points without assuming independence between points or equal correlations over time (14). It compares treatment groups in terms of both the overall level of heart rate (constant term) and the shape of the time trends. The shape of the time trends are modeled on a third degree orthogonal polynomial, thus allowing comparisons of the linear rate of change (x term) and the pattern of response (x^2 and x^3 terms) between groups. All data are expressed as the mean±SD.

Results

Effect of intrathecal T_3 (Fig. 1). All rats received either T_3 or vehicle intrathecally. The heart rate did not change in response to vehicle (base-line heart rate: 332±29 beats per minute (bpm) after intrathecal NaOH: 331±21 bpm). In T_3-treated rats, the heart rate increased significantly from 326±24 bpm before to 343±25 bpm after intrathecal administration ($P < 0.02$). Comparison of results in T_3-treated vs. vehicle-treated animals revealed significant differences in both the overall heart rate level (F = 8.5, degrees of freedom (df) = 1, 8; $P < 0.02$) and the rate of heart rate change over time (F = 5.0, df = 1, 8; $P < 0.05$). The results indicate that T_3 enhanced heart rate overall and that this occurred in a linearly increasing fashion. This is apart from the diurnal pattern of heart rate response which is apparent and similar in both groups.

Effect of other iodothyronines (Fig. 2). To exclude the possibility that the results after intrathecal T_3 were due to nonspecific heart rate-stimulating effects of intrathecally administered iodothyronine, a three-way comparison of heart rates was carried out after administration of 1.5 nmol/100 g body wt of either T_4, rT_3, or NaOH. The heart rate did not change significantly in any group and pre- and postinjection values were not significantly different among groups (pre- and postinjection values, respectively: T_4, 329±27 and 339±22 bpm; rT_3, 308±21 and 301±30 bpm; NaOH, 322±29 and 327±16 bpm).

Effect of intravenous T_3 (Fig. 3). Additional experiments were conducted to confirm the intrathecal T_3 effect and to determine the heart rate response in intravenous T_3-treated animals when handled and evaluated by the methods used in these investigations. A four-way comparison of heart rate was carried out after administration of T_3 or vehicle by the intrathecal or intravenous route. A diurnal variation in heart rate response was not observed in any of four groups of rats studied in this experiment, although it was observed in the groups studied in the first and second experiments (see Figs. 1 and 2). The reason for this difference is unclear.

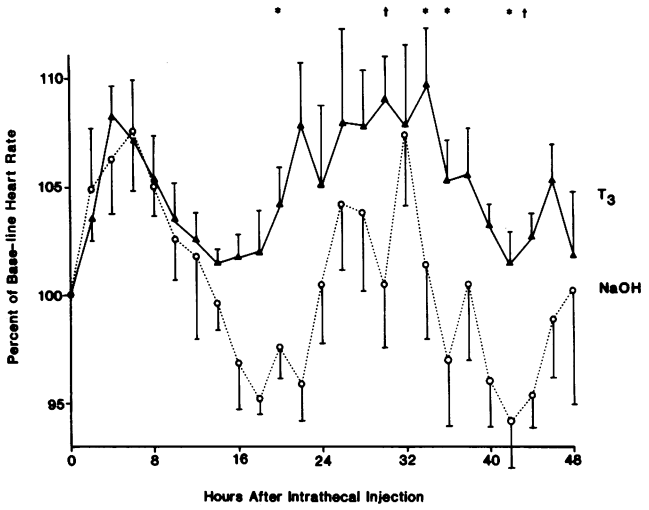

Figure 1. Fractional heart rate changes after intrathecal administration of T_3 or vehicle. Hypothyroid rats were prepared and monitored as described in Methods. Mean heart rates for each 2-h postinjection period is expressed as a fraction of the mean base-line heart rate±SD. The overall difference between vehicle-treated and T_3-treated rats was significant ($P < 0.02$); t test at each time interval showed significant differences as indicated by *($P < 0.05$) or †($P < 0.02$); n = 5.

Base-line and posttreatment heart rates, respectively, were: 372±26 and 369±34 bpm, intrathecal NaOH; 370±34 and 394±27 bpm, intrathecal T_3; 382±34 and 358±43 bpm, intravenous NaOH; 380±24 and 379±25 bpm, intravenous T_3. Multivariate analysis of variance for repeated measures showed an overall increase over base-line heart rates only in rats given

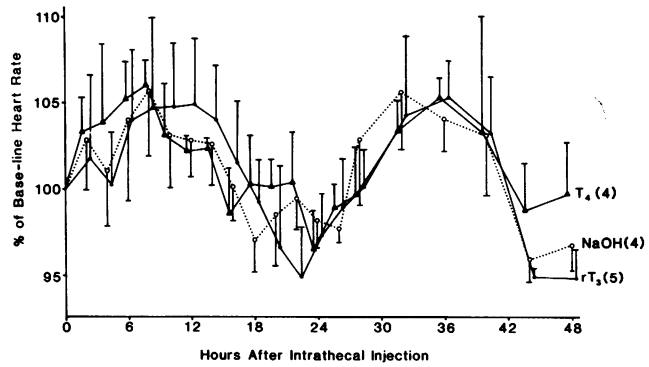

Figure 2. Mean fractional heart rate changes±SD after intrathecal administration of 1.5 nmol T_4 or rT_3/100 g body wt or vehicle to rats prepared and monitored as described in experiment 1. No significant heart rate change over base line was noted in any group; overall differences in postinjection heart rates among rats given T_4, rT_3, or NaOH were not significant. · · ·○· · ·, NaOH; – ● –, rT_3; ▲, T_4.

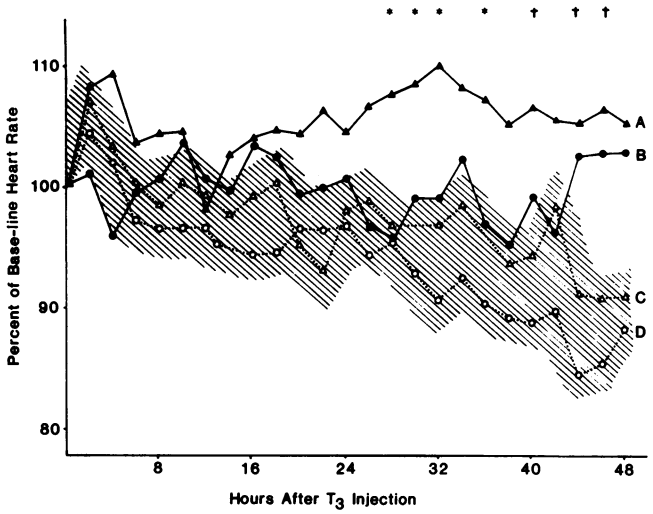

Figure 3. Effect of route of administration on fractional heart rate response to T_3. Pairs of rats receiving 1 μg T_3/100 g body wt or NaOH by intravenous or intrathecal injection were observed simultaneously. Mean fractional changes from base line are shown in two hourly intervals after intrathecal T_3 (A, – ▲ –), intravenous T_3 (B, – ● –), intrathecal NaOH (C, · · · △ · · ·), or intravenous NaOH (D, · · · ○ · · ·); shaded area shows composite range of SD for NaOH-treated groups which were not significantly different from each other. Fractional heart rate responses in intrathecally T_3-treated rats were significantly greater than in those given intravenous T_3 ($P < 0.04$) or in NaOH-treated groups separately or combined ($P < 0.02$); t test revealed significantly increased rates after intrathecal T_3 relative to intrathecal NaOH at time intervals shown by *($P < 0.05$) or †($P < 0.02$); $n = 6$ in each category.

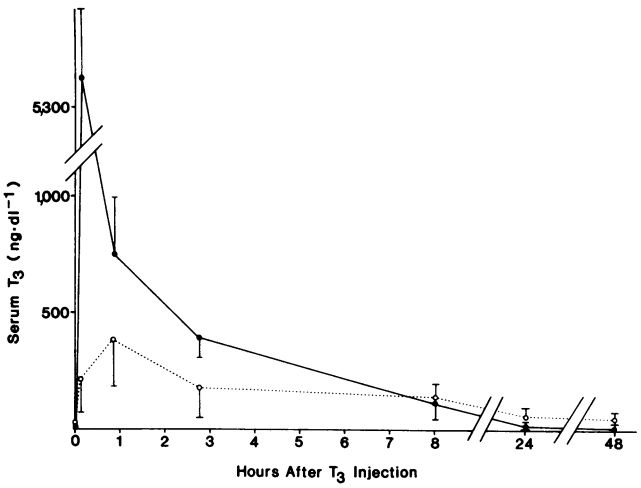

Figure 4. Serum T_3 concentrations following intravenous or intrathecal T_3 administration. Base-line T_3 values of the two groups were in the hypothyroid range and were not significantly different from each other. Differences between the groups were significant at 5, 50, and 150 min but not thereafter; at 24 and 48 h, serum T_3 levels had returned to base line in the intravenous T_3-injected group but were still significantly above base line in the intrathecally T_3-injected animals. Integrated T_3 values were significantly higher by 40% in rats receiving the hormone intravenously ($P < 0.01$) as compared with rats treated by the intrathecal route.

intrathecal T_3; these increases were established by 18 h after injection and were significant whether compared with rates after intrathecal NaOH ($F = 8.3$, df = 4, 10; $P < 0.01$) or intravenous T_3 ($F = 5.3$, df = 4, 10; $P < 0.04$). No significant heart rate change was found in the rats given NaOH and no significant differences were noted between rats receiving NaOH by the intravenous vs. the intrathecal route ($P < 0.24$). Though overall heart rate responses of rats given intravenous T_3 were not significantly different from those given intravenous NaOH ($P < 0.13$), the possibility that a delayed effect of intravenous T_3 would have appeared if the experiment had been prolonged beyond 48 h was suggested by a linear trend toward increased heart rate which approached significance ($P < 0.08$).

Serum T_3 levels after intravenous and intrathecal T_3 administration (Fig. 4). To determine the rapidity and extent that intrathecally administered T_3 appears in the bloodstream, we compared serum T_3 levels (radioimmunoassay) in a separate group of matched hypothyroid rats given T_3 by the intravenous or intrathecal route. Time-integrated serum T_3 values were significantly higher in the intravenous-treated animals ($P < 0.01$). Therefore, the heart and other peripheral tissues were exposed to significantly higher serum concentrations of T_3 after intravenous than after intrathecal T_3 administration.

Discussion

Thyroid hormones pass from blood to brain by a carrier-mediated mechanism with affinity for T_3 which is reported to exceed that of any known hormone transport system operating across the blood-brain barrier (2). However, the capacity of this system is small, and as a result, T_3 in the systemic circulation equilibrates very slowly with brain, with a half-time between 3 and 6 h (3). Therefore, centrally mediated effects of T_3 reaching the brain through the systemic circulation are likely to be delayed as compared with T_3 effects in peripheral tissues. We have observed that T_3 administration by the intrathecal route results in a faster rate of uptake and a greater concentration of hormone within the brain as compared with results after intravenous administration (data not shown). While intrathecal injection does not guarantee more efficient or rapid delivery to any particular brain region, circumventing the blood-brain barrier by using the intrathecal route has proven to be useful in promoting brain uptake of many compounds (15).

The T_3 dose used in these experiments (1,000 ng/100 g) is higher than considered physiologic (serum T_3 production rate in the rat is ~200–250 ng/100 g per d). However, functional responses of hypothyroid subjects to a physiologic thyroid hormone dose usually follows repeated administration of that amount. When the response to a single dose of T_3 is being evaluated, it is usual to give a dose at an order of magnitude greater than we gave in these experiments (for examples, see references 11, 16, and 17). Evidence that we chose an appropriate dose for these investigations is indicated by the responses themselves. A sensitive indicator of T_3 effects, heart rate, did not change significantly when 1.5 nM T_3 was given intravenously, whereas a small but significant heart rate change was caused by intrathecal injection of the same amount. It would have been interesting to observe the response to even larger amounts of T_3, but the dose was limited by the solubility of the hormones and the requirement to inject no more than a 4-μl volume into the lateral cerebral ventricle.

Two previous reports have shown that a significant increase in core temperature occurs following intrathecal T_4 administra-

tion (18, 19). While the evidence is compatible with a central nervous system mechanism of T_4 action, the experiments were not controlled so as to examine this point specifically. We chose to study effects of central nervous system iodothyronines on heart rate because this function is (*a*) dependent not only on peripheral mechanisms but also on mechanisms integrated and controlled in the brain (20); (*b*) markedly susceptible to changes in thyroid hormone availability (21); (*c*) stimulated by thyroid hormones at least in part through a nervous system-related mechanism (22); and (*d*) susceptible to quantitative measurements. Under the conditions of these experiments, no heart rate change was observed after intrathecal T_4 or rT_3, indicating that the response measured was specific to T_3. We ascribe the greater (or earlier) heart rate change observed after intrathecal as compared with intravenous T_3 to its earlier uptake and higher concentration in T_3-responsive centers in brain.

While the data reported here point to a T_3-responsive central nervous system mechanism in heart rate regulation, the results in no way detract from the heart rate-stimulating effects of T_3 exerted within the neural or myocardial components of the heart itself. Rather, bidirectional passage of iodothyronines across the blood-brain barrier (23) would make it likely that both central and peripheral iodothyronine-dependent processes are involved in regulating cardiovascular dynamics. Though the cellular mechanism is still unknown, the information provided by this study adds to existing biochemical and autoradiographic evidence (3, 5, 6) supporting a neuroregulatory role for T_3 or its metabolites within the central nervous system.

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References

1. Dratman, M. B., F. L. Crutchfield, J. T. Gordon, and A. S. Jennings. 1983. Iodothyronine homeostasis in rat brain during hypo- and hyperthyroidism. *Am. J. Physiol.* 245:E185–E193.

2. Pardridge, W. M. 1979. Carrier-mediated transport of thyroid hormones through the rat blood-brain barrier: primary role of albumin-bound hormone. *Endocrinology.* 105:605–612.

3. Dratman, M. B., and F. L. Crutchfield. 1978. Synaptosomal [^{125}I]triiodothyronine following intravenous [^{125}I]thyroxine. *Am. J. Physiol.* 4:E638–647.

4. Kaplan, M. M., and K. A. Yaskoski. 1980. Phenolic and tyrosyl ring deiodination of iodothyronines in rat brain homogenates. *J. Clin. Invest.* 66:551–562.

5. Dratman, M. B., Y. Futaesaku, F. L. Crutchfield, N. Berman, B. Payne, M. Sar, and W. E. Stumpf. 1982. Iodine 125-labelled triiodothyronine in rat brain: evidence for localization in discrete neural systems. *Science (Wash. DC).* 215:309–312.

6. Dratman, M. B., F. L. Crutchfield, J. Axelrod, R. W. Colburn, and N. Thoa. 1976. Localization of triiodothyronine in nerve ending fractions of rat brain. *Proc. Nat. Acad. Sci. USA.* 73:941–944.

7. Schwartz, H. L., and J. H. Oppenheimer. 1978. Nuclear triiodothyronine receptor sites in brain: probable identity with hepatic receptors and regional distribution. *Endocrinology.* 103:267–273.

8. Mashio, Y., M. Inada, K. Tanaka, H. Ishii, K. Naitro, M. Nishikowa, K. Takahashi, and H. Imura. 1982. High affinity 3,5,3'-L-triiodothyronine binding to synaptosomes in rat cerebral cortex. *Endocrinology.* 110:1257–1261.

9. Riuz-Marcos, A., F. Salas, F. Sanchez-Toscano, F. Escobar del Rey, and G. Morreale de Escobar. 1983. Effect of neonatal and adult-onset hypothyroidism on pyramidal cells of the rat auditory cortex. *Dev. Brain Res.* 9:205–213.

10. Emlen, W., D. S. Segal, and A. J. Mandell. 1972. Thyroid state: effects on pre- and postsynaptic central noradrenergic mechanism. *Science (Wash. DC).* 175:79–82.

11. Bray, G., and H. M. Goodman. 1965. Studies on the early effects of thyroid hormones. *Endocrinology.* 76:323–328.

12. Meselis, R. R., and A. N. Epstein. 1975. Feeding induced by intracerebroventricular 2-deoxy-D-glucose in the rat. *Am. J. Physiol.* 229: 1438–1444.

13. Jennings, A. S., D. C. Ferguson, and R. D. Utiger. 1979. Regulation of the conversion of thyroxine to triiodothyronine in the perfused rat liver. *J. Clin. Invest.* 64:1614–1623.

14. Bock, R. D. 1975. Multivariate Statistical Methods in Behavioral Research. McGraw Hill, New York. 449–489.

15. Myers, R. D. 1974. Handbook of Drug and Chemical Stimulation of Brain. Behavioral, Pharmacological, and Physiological Aspects. Van Nostrand Reinhold Co., New York.

16. Leonard, J. L., M. M. Kaplan, T. J. Visser, J. E. Silva, and P. R. Larsen. 1981. Cerebral cortex responds rapidly to thyroid hormone. *Science (Wash. DC).* 214:571–573.

17. DeGroot, L. J., P. Rue, M. Robertson, J. Bernal, and N. Scherberg. 1977. Triiodothyronine stimulates nuclear RNA synthesis. *Endocrinology.* 101:1690–1700.

18. Belesin, D. B., and R. Samardzic. 1973. Effect of thyroxine on the body temperature after its intraventricular injection into conscious cats. *J. Physiol.* 238:27P–28P.

19. Kaciuba-Uscilko, H., J. Sobocinski, S. Kozlowski, and A. W. Ziemba. 1976. Effect of intraventricular thyroxine administration on body temperature in dogs at rest and during physical exercise. *Experientia (Basel).* 32:351–352.

20. Korner, P. I. 1971. Integrative neural cardiovascular control. *Physiol. Rev.* 51:312–367.

21. Pietros, R. J., M. A. Real, G. S. Poticha, D. Bronsky, and S. Waldstein. 1972. Cardiovascular response in hyperthyroidism. *Arch. Intern. Med.* 129:426–429.

22. Dratman, M. B. 1978. The mechanism of thyroxine action. *In* Hormonal Proteins and Peptides. C. H. Li, editor. Academic Press, New York. 238–257.

23. Chernow, B., K. D. Burman, D. L. Johnson, R. A. McGuire, J. T. O'Brien, L. Wartofsky, and L. P. Georges. 1983. T_3 may be a better agent than T_4 in the critically ill hypothyroid patient: evaluation of transport across the blood-brain barrier in a primate model. *Critical Care Med.* 11:99–104.