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Thyroid Hormones Directly Interact with Vascular Smooth Muscle Strips

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SUMMARY

The thyroid hormones have direct effects on vascular smooth muscle and are potent vasorelaxants. In the present study, the effects of *d*- and *l*-thyroxine (*d*-T_4 and *l*-T_4), 3,5,3′-triiodo-*d*-thyronine (*d*-T_3), and 3,5,3′-triiodo-*l*-thyronine (*l*-T_3) on the isolated mesenteric artery of the rabbit and superprecipitation of actomyosin from bovine aorta were examined. These thyroid hormones dose dependently relaxed vascular strips previously contracted with 50 mM KCl in the presence of phentolamine (1 μM), propranolol (1 μM), and atropine (0.3 μM), and the order of the inhibitory potency was *l*-T_4 > *d*-T_4 > *l*-T_3 > *d*-T_3 for the contraction. Pretreatment with *l*-T_4 (10 and 30 μM) inhibited the contractile response concomitant with the inhibition of the 20,000-Da myosin light chain phosphorylation, without significant suppression of the increase in La^{3+}-resistant ^{45}Ca influx and uptake (5 and 30 min) induced by 50 mM KCl, suggesting that the inhibitory effect of *l*-T_4 may not be primarily related to Ca^{2+} entry through the voltage-dependent Ca^{2+} channel. The *l*-T_4 (10 and 30 μM) showed noncompetitive antagonism against the Ca^{2+}-induced contraction in the high K^+-depolarized vascular strips. Superprecipitation of actomyosin was inhibited by the addition of *l*-T_4, in a dose-dependent manner, and calmodulin (1 μg/ml) partly reversed the inhibitory effect of *l*-T_4. Thyroid hormones were found to inhibit Ca^{2+}/calmodulin-dependent smooth muscle myosin light chain kinase, and the K_i value for *l*-T_4 was 2.5 μM. Although the concentrations of *l*-T_4 used in this study are high, relative to circulating physiological levels, thyroid hormones act directly at the blood vessel wall to cause inhibition of the contractile process in vascular smooth muscle *in vitro*. Modulation of the 20,000-Da myosin light chain phosphorylation via the inhibition of myosin light chain kinase activity may at least in part contribute to the inhibitory effect of *l*-T_4.

It is well known that the cardiovascular functions can be influenced by diverse factors, especially those that are mainly regulated through the autonomic nervous system and through hormones in the circulation. Several reports have revealed that the thyroid hormone is related to β-adrenergic receptor-mediated cardiovascular mechanical responses. In vascular smooth muscle, Parker *et al.* (1) suggested that thyroid hormones are necessary for β-adrenoceptor-mediated relaxation. Recently, β1-adrenoceptors appeared to be functionally predominant in vascular strips from T_4-treated animals (2). Furthermore, it has very recently been reported that modulation of the cardiovascular responsiveness to β-stimulation by thyroid hormone is different in aged animals (3). Furthermore, in cultured rat atrial myocytes, it has been shown that the thyroid hormones directly stimulated synthesis of atrial natriuretic peptide (4).

There is, however, little information available concerning the direct interaction of thyroid hormones with the actomyosin system. The present study was undertaken to determine whether thyroid hormones possess a direct effect on the isolated vascular smooth muscle. In this communication, the effects of thyroid hormones on isometric tension development, myosin light chain phosphorylation, and ^{45}Ca uptake in intact rabbit vascular strips were studied *in vitro*. The effect of thyroid hormones on superprecipitation of actomyosin from bovine aorta was also investigated.

Materials and Methods

Preparation of vascular strips. Experiments were performed on superior mesenteric artery taken from male albino rabbits, weighing about 2.5 kg, after exsanguination during anesthesia with sodium pentobarbital (30 mg/kg intravenously). The blood vessel (0.8–1.8 mm outside diameter) was cleaned of excess fat and adherent connective tissue and cut into helical strips of 1 × 10 mm (for tension recording) or 1.5 × 20 mm (for biochemical analysis).

Recording of mechanical responses. The vascular strips were vertically suspended in a 20-ml organ bath filled with Krebs-Henseleit solution of the following composition (mM): NaCl, 115; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; and dextrose, 10. The organ bath was kept at 37° (pH 7.4) and aerated with 95% O_2/5% CO_2. The mechanical activity was recorded isometrically by means of a force-displacement transducer (TB-612T; Nihon-Kohden Kogyo Co.,

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ABBREVIATIONS: T_4, thyroxine; MOPS, 3-(*N*-morpholino)propanesulfonic acid; LC_{20}, 20,000-Da myosin light chain; MLCK, myosin light chain kinase; CaM, calmodulin; EGTA, CaM kinase II, calmodulin-dependent protein kinase II; T_3, 3,5,3′-triiodothyronine.

Ltd., Tokyo, Japan). The preparations were stretched progressively to the optimal point (approximate basal tension, 0.5 g). Before initiation of the experiments, the tissues were allowed to equilibrate for 1 hr in the Krebs-Henseleit solution. In all experiments, precontraction was produced twice by the addition of 50 mM KCl before a dose-response curve was determined.

Two protocols were used to examine the influence of thyroid hormones on tension development and tension maintenance. In the first protocol, to demonstrate the relaxing effect of thyroid hormones, strips were contracted by KCl (50 mM) before challenge with thyroid hormones. Cumulative dose-relaxation curves for *l*-T_4 and other thyroid hormones against the high K^+-induced contraction were obtained, as described previously (5, 6). Papaverine at a concentration of 100 μM was added at the end of each series of experiments and the relaxation induced by papaverine was taken as 100%. When determining the ED_{50} values, we calculated the response to thyroid hormones as a percentage of the maximum relaxation obtained. The ED_{50} value was obtained visually from a plot of percent relaxation versus log concentration of a contraction and was observed as follows. In control tissues, responses to 50 mM KCl were repeated at 50-min intervals to test for reproducibility. In other preparations, *l*-T_4 or *d*-T_4 (10 or 30 μM) was added 20 min after the first response to KCl was obtained, and the reaction was allowed to proceed for 30 min before the KCl challenge was repeated. The initial peak amplitude of the first contraction was compared with that of the second contraction.

The antagonistic effect of *l*-T_4 on the Ca^{2+}-induced contraction in depolarized muscle was examined using much the same procedures as those described by Ishikawa *et al.* (6).

Determination of LC_{20} phosphorylation. The state of phosphorylation of LC_{20} was determined on glycerol/acrylamide slab gels (7). Experimental procedures were performed according to previously described methods (8).

La^{3+}-resistant Ca^{2+} uptake and unidirectional Ca^{2+} influx. Cellular Ca^{2+} content was measured according to slight modifications of the method of Karaki and Weiss (9) and Asano and Hidaka (10). Briefly, helical strips of rabbit mesenteric artery were placed into glass scintillation vials containing 5 ml of physiological salt solution of the following composition (in mM): NaCl, 140; KCl, 5.9; $CaCl_2$, 1.5; $MgCl_2$, 1.2; dextrose, 10.0, and MOPS, 5.0 (pH 7.2). During the course of the ^{45}Ca experiments, the MOPS-buffered solutions were aerated with 100% O_2. The preparations were equilibrated for 60 min and then transferred to a high K^+ (50 mM) solution with added ^{45}Ca (specific activity, 0.8 μCi/ml). *l*-T_4 (30 μM) or nifedipine (0.3 μM) were included during the entire incubation time (30 min, La^{3+}-resistant Ca^{2+} uptake). After the 30-min exposure to this ^{45}Ca solution with and without *l*-T_4 or nifedipine, the tissues were transferred into the vials containing 20 ml of ice-cold (0.5°) La^{3+}-substituted solution that contained 80.8 mM $LaCl_3$, 10 mM dextrose, and 5 mM MOPS, adjusted to pH 7.2 with 6 N NaOH, for sequential periods of 1, 29, and 30 min (total 60-min rinse). The strips were then removed from the La^{3+}-substituted solution, gently blotted with No. 2 Toyo filter paper (Toyo Roshi Co., Ltd., Tokyo, Japan), and weighed on a Mettler AE160 balance (Mettler Instrument Corp., Highstown, NJ). Each strip was then placed in a glass scintillation vial containing 0.5 ml of Protosol (NEN tissue solubilizer; E. I. du Pont de Nemours & Co.) and solubilized at 45° for 12 hr. Solubilized muscles were neutralized with acetic acid and mixed with 12 ml of scintillation cocktail (ACS-II; Amersham, Arlington Heights, IL).

Using protocols and solutions similar to those described above, the effects of *l*-T_4 and nifedipine on unidirectional ^{45}Ca influx (11) to La^{3+}-resistant sites were quantitated by shortening the duration of exposure to the 1.5 mM Ca^{2+} plus ^{45}Ca solution to 5 min rather than 30 min. Additionally, *l*-T_4 and nifedipine were added 30 min before and during the 5-min exposure to the labeled solution. Otherwise, the preexposure and La^{3+} rinse procedures were exactly the same as above.

Values for cellular Ca^{2+} content (expressed as μmol/kg of muscle wet weight) could be calculated by multiplying the ^{45}Ca tissue/medium ratio by the concentration of Ca^{2+} (μmol/liter) in the radioactive incubation solution (9).

The effect of *l*-T_4 on cellular Ca^{2+} content was compared with that of nifedipine, a Ca^{2+} channel blocker.

Preparations for enzyme assay. Myosin light chain was prepared by the method of Perrie and Perry (7). MLCK was purified from chicken gizzard by the method of Adelstein and Klee (12). CaM was purified from bovine brain by the method of Endo *et al.* (13). CaM kinase II was purified from rabbit brain by a slight modification of procedure described by Kennedy *et al.* (14).

Enzyme assay and determinations. MLCK and CaM kinase II activities were assayed under the conditions described earlier (15), in a reaction mixture containing, in a final volume of 0.2 ml, 50 mM Tris·HCl (pH 7.0), 10 mM magnesium acetate, 0.1 mM $CaCl_2$ or 1 mM EGTA, 100 ng of CaM, 5 to 100 μM [γ-^{32}P]ATP (4×10^5 cpm), 20 μM smooth muscle LC_{20}, and 0.6 μg of MLCK or CaM kinase II. The incubation was carried out at 30° for 5 min. The reaction was terminated by the addition of 1 ml of ice-cold 20% trichloroacetic acid following the addition of 500 μg of bovine serum albumin as a carrier protein. The sample was centrifuged at 3000 rpm for 15 min, the pellet was resuspended in ice-cold 10% trichloroacetic acid solution, and the centrifugation-resuspension cycle was repeated three times. The final pellet was dissolved in 1 ml of 1 N NaOH and radioactivity was measured in liquid scintillation counter.

Experiments using bovine aorta smooth muscle actomyosin. Actomyosin was prepared from bovine aorta smooth muscle by the methods of a previous report (5, 16). Superprecipitation of actomyosin (7.0 mg of protein/ml) was followed by an increase in A_{660} at 25° after addition of 2 mM Mg-ATP in the presence of 20 mM MOPS, 50 mM KCl, and 11 μM $CaCl_2$ at pH 7.0 in a total volume of 3 ml.

Drugs and chemicals. The drugs used were *d*- and *l*-T_4 (Sigma Chemical Co., St. Louis, MO), *d*- and *l*-T_3 (Sigma), nifedipine (Bayer), phentolamine mesylate (Regitine mesylate; Ciba-Geigy), *dl*-propranolol hydrochloride (Sigma), atropine sulfate (Wako), and papaverine hydrochloride (Wako).

Results

Effects of thyroid hormones on K^+-induced contractions. Strips of rabbit mesenteric arteries with or without endothelium were studied in the presence of phentolamine (1 μM), propranolol (1 μM), and atropine (0.3 μM), to block α- and β-adrenoceptors and cholinergic muscarinic receptors, respectively. The presence or absence of functional endothelium was determined at the start of each experiment by the relaxation response of KCl (50 mM)-precontracted strips to acetylcholine (17). The addition of KCl at a concentration of 50 mM caused a near maximal sustained contraction in the preparations. When the contraction was at steady state level, thyroid hormones (1 to 100 μM) were added to the organ bath. Cumulative application of thyroid hormones, i.e., *l*- or *d*-T_4 and *l*- and *d*-T_3) caused concentration-dependent relaxations. Typical responses of mesenetric arterial strips to *l*-T_4 are shown in Fig. 1a. The relaxing effects of thyroid hormones on the unrubbed or rubbed arterial strips were not significantly different and were not affected significantly by incubation with indomethacin (10 μM) (data not shown). Concentrations of the thyroid hormones needed to produce 50% relaxation of the contraction (ED_{50}) in mesenteric artery are listed in Table 1. The descending order for intensity of relaxing activities was *l*-T_4 > *d*-T_4 > *l*-T_3 > *d*-T_3.

In addition, pretreatment with *l*-T_4 for 30 min dose-dependently inhibited the development of tension in response to 50 mM KCl (Fig. 1b). In contrast to the marked effects of *l*-T_4 on sustained contraction, the tension development was less af-

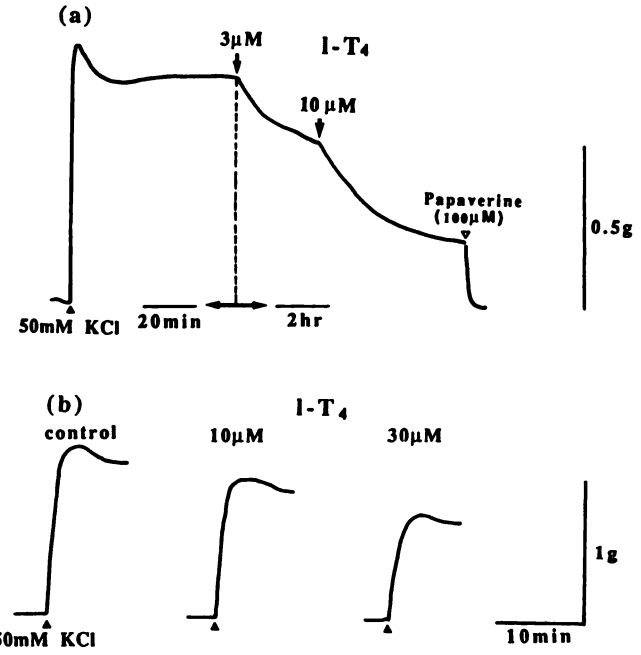

Fig. 1. Responses of strips of rabbit mesenteric artery to *l*-T_4 or *d*-T_4. a, Relaxing effect of *l*-T_4 or *d*-T_4 on the tonic contraction produced by 50 mM KCl. b, Inhibition of KCl (50 mM) tension development by pretreatment with *l*-T_4 or *d*-T_4. See Materials and Methods for details.

TABLE 1

Relaxing effect of thyroid hormones on isolated rabbit mesenteric arterial strips contracted by 50 mM KCl

Data are expressed as mean ± standard error. *n* indicates the number of preparations used. See Materials and Methods for details.

Thyroid hormone	Vascular relaxation, ED_{50}	*n*
	μM	
l-T_4	7.6 ± 0.7	8
d-T_4	15 ± 1.8	7
l-T_3	41 ± 10	4
d-T_3	83 ± 6.8	4

fected at the two concentrations of *l*-T_4 examined. The same results were also obtained with *d*-T_4 (data not shown). Because of the insolubility of *l*-T_4 and *d*-T_4, the experiment could not be carried out at concentrations higher than 30 μM.

Inactive analogues such as 3,5-diiodo-*l*-tyrosine, *l*-thyronine, 3,5-diiodo-*l*-tyrosine, or *d*-, *l*-tyrosine had no marked effect on the vascular strips contracted by 50 mM KCl at up to 30 μM (data not shown).

Effect of *l*-T_4 on Ca^{2+}-induced contraction in high K^+-depolarized arterial strips. To examine further the inhibitory effect of thyroid hormones, the effect of *l*-T_4 on the Ca^{2+}-induced contraction of depolarized rabbit mesenteric artery was also studied. As shown in Fig. 2, *l*-T_4 inhibited, concentration dependently, the Ca^{2+}-induced contraction, producing a shift of the dose-response curve both rightwards and downwards, indicative of a noncompetitive antagonism. This result indicates that *l*-T_4 may not be a Ca^{2+} channel blocker. This idea was confirmed by the Ca^{2+} uptake experiment using ^{45}Ca, as described below.

Effect of *l*-T_4 on La^{3+}-resistant Ca^{2+} uptake and influx. As shown in Fig. 3, high K^+ (50 mM) increased the $^{45}Ca^{2+}$ influx or uptake during 5- or 30-min incubation periods in rabbit mesenteric artery. Parallel experiments were carried out to

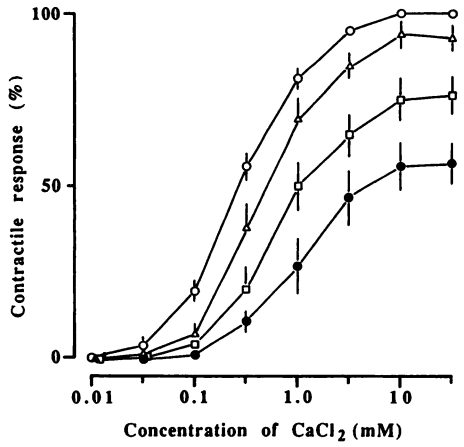

Fig. 2. Influence of *l*-T_4 on the dose-response curves for $CaCl_2$ in rabbit mesenteric artery. Each preparation was contracted in Ca^{2+}-free, 80 mM K^+ Krebs-Henseleit solution. 0 (○), 3 (△), 10 (□), or 30 μM (●) *l*-T_4 was added 30 min before the addition of $CaCl_2$. In each preparation, the maximum contraction developed in response to $CaCl_2$ in the control solution was taken as 100%. Shown are the mean values and their standard deviations, as vertical bars (four experiments).

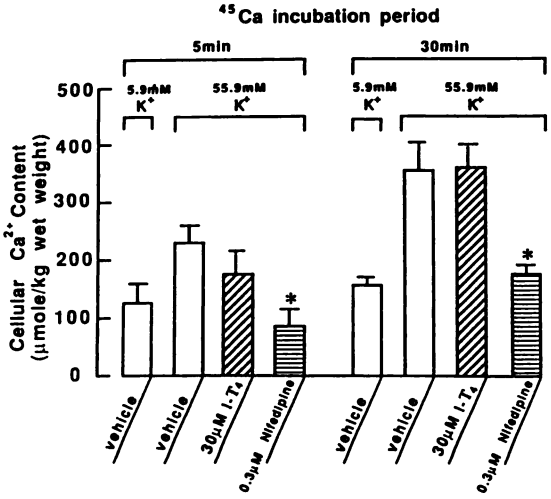

Fig. 3. Effects of *l*-T_4 and nifedipine on the high K^+ challenge-induced increase of cellular Ca^{2+} content in the rabbit mesenteric arterial strips. Helical strips of rabbit mesenteric artery were incubated with $^{45}Ca^{2+}$ for either 5 or 30 min in either normal (5.9 mM) or high (55.9 mM) K^+ bathing solutions before a 60-min washout in La^{3+}-substituted solution (for details, see Materials and Methods). The *l*-T_4 or nifedipine was added 30 min before and also during the $^{45}Ca^{2+}$ incubation period. Data are expressed as mean ± standard error (four experiments). *Significantly different when compared with each control ($p < 0.05$).

study the effects of *l*-T_4 or nifedipine on net Ca^{2+} uptake and unidirectional Ca^{2+} influx induced by 50 mM KCl.

The treatment of rabbit mesenteric arterial strips with 30 μM *l*-T_4 produced no significant effect on the 30-min ^{45}Ca incubation value of the high K^+-stimulated increase in cellular Ca^{2+} content (Fig. 3). Nifedipine at a concentration of 0.3 μM reduced the 30-min $^{45}Ca^{2+}$ incubation value of the high K^+-stimulated increase in cellular Ca^{2+} content (Fig. 3).

Monitoring Ca^{2+} influx for short periods of incubation time (5 min) has been used as an indication of the rate of Ca^{2+} influx, to minimize the effect of the complicating influence of Ca^{2+} efflux. The increase in unidirectional Ca^{2+} influx induced by high K^+ was significantly inhibited by nifedipine (0.3 μM) but not by *l*-T_4 (30 μM) at concentrations that had inhibitory

effects on KCl-induced contraction. It is suggested that *l*-T_4 antagonizes the contractile response of vascular smooth muscle without preventing the transport of Ca^{2+} via cell membrane.

Effect of *l*-T_4 on LC_{20} phosphorylation. Changes in phosphate content in the myosin molecule during contraction were studied by contracting rabbit mesenteric arterial strips with high K^+. The data in Fig. 4 show the effect of *l*-T_4 pretreatment on LC_{20} phosphorylation and contraction elicited by 50 mM KCl. The strips were exposed to 30 μM *l*-T_4 for 30 min before stimulation with KCl. The contractile response to KCl depolarization reached a maximum at about 2 min and was maintained up to at least 2 hr (data not shown) whereas LC_{20} phosphorylation declined to near basal levels within 2 min. LC_{20} phosphorylation reached a maximum of 5.9 ± 0.05 mol of P_i/mol of LC_{20} 10 sec after the addition of KCl. The initial peak levels of LC_{20} phosphorylation at 10 sec were significantly depressed by *l*-T_4 (0.24 ± 0.08 mol of P_i/mol of LC_{20}). LC_{20} phosphorylation and contraction were inhibited by *l*-T_4.

Effect of *l*-T_4 on superprecipitation of bovine aorta actomyosin. When 2 mM ATP was added to the reaction mixture containing bovine aorta actomyosin, superprecipitation of actomyosin occurred and this was determined by measuring the increase in absorbance at 660 nm (Fig. 5a). Addition of *l*-T_4 produced inhibition of superprecipitation of the actomyosin in a dose-dependent manner (data not shown). *l*-T_4 (30 μM) inhibited the extent of superprecipitation and prolonged the time required to attain a maximum level of superprecipitation (Fig. 5b). The inhibitory effect of *l*-T_4 was reversed by the addition of 1 μg/ml CaM (Fig. 5c). However, the addition of 1 μg/ml CaM alone had little effect on the superprecipitation (Fig. 5d). The result is consistent with the data showing that the inhibition of MLCK by *l*-T_4 was competitive with respect to CaM (18).

***In vitro* effects of thyroid hormones.** Effect of thyroid hormones on the activities of two Ca^{2+}/CaM-dependent protein kinases, MLCK and CaM kinase II, were examined and the data are summarized in Table 2. As listed in Table 2, thyroid hormones inhibited the MLCK activity in a concentration-dependent manner and the IC_{50} values of *l*-T_4, *d*-T_4, *l*-T_3, and *d*-T_3 are 8.9, 7.8, 22, and 15 μM, respectively. Conversely, thyroid hormones had a weak effect on CaM kinase II. In the case of *l*-T_4, the ability to inhibit Ca^{2+}/CaM-activated MLCK activity was correlated with its inhibitory effect on KCl-mediated sustained contraction (Tables 1 and 2).

Discussion

The results of this study show that thyroid hormones, particularly *l*-T_4, have a direct inhibitory effect on vascular contraction. In our study, *l*-T_4 itself causes vascular relaxation in the presence of the β-receptor-blocking drug propranolol, suggesting that *l*-T_4 does not act through the β-receptor-associated mechanism(s). Moreover, the relaxation induced by *l*-T_4 was not affected by treatment with the α-adrenergic receptor blocker phentolamine or the muscarinic cholinergic blocking agent atropine. Because the effects of *l*-T_4 on the unrubbed or rubbed vascular strips, as well as indomethacin-treated arterial strips, were not significantly different, the release of endothelium-derived relaxing factor(s) or the production of prostacyclin may also be ruled out in *l*-T_4-induced vascular relaxation.

Thyroid hormones are known to interact with intracellular and/or nuclear receptors (to bind thyroxine-binding globlin), triggering the effect. In this study, high concentrations of thyroid hormones may possess direct modes of action in smooth muscle cells; thus, these hormones exert relatively rapid action at isolated vascular strips. The *l*-T_4-induced relaxation is not due to membrane receptor-associated mechanisms; rather, it has an effect on the intracellular site(s) and common events in the contractile response of vascular smooth muscle.

Ca^{2+}/CaM-dependent phosphorylation of the LC_{20} catalyzed by MLCK is thought to be important for tension development (19, 20). Pharmacological regulation of smooth muscle contractile activity may occur through modulation of the LC_{20} phosphorylation system (21). Drugs thought to inhibit contractility in cardiac and smooth muscle by selective inhibition of Ca^{2+} channels, so-called Ca^{2+} channel blockers, indirectly modulate the LC_{20} phosphorylation. Limitation of Ca^{2+} accessibility to MLCK and contractile proteins is their mode of action. One possible site of action for *l*-T_4 would be inhibition of Ca^{2+} entry through the voltage-dependent Ca^{2+} channel. However, this possibility is not likely because the cellular ^{45}Ca content of high

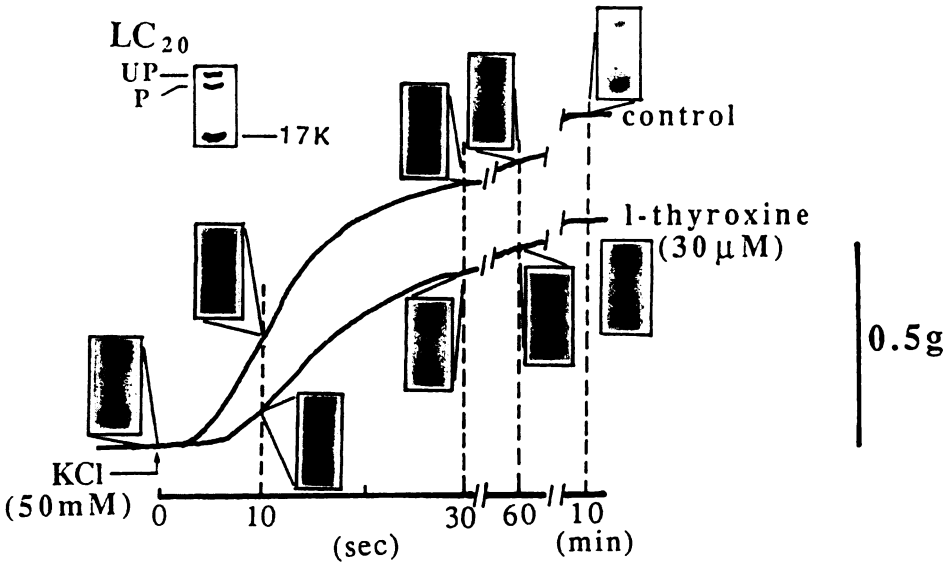

Fig. 4. Response of the rabbit mesenteric artery to *l*-T_4. Vascular strips were frozen during rest or at indicated times between 10 and 30 sec of stimulation. The preparation was contracted with 50 mM KCl. The *l*-T_4 was added 30 min before the addition of KCl. The *insets* show urea gel electrophoresis. Phosphorylation was measured as described as under Materials and Methods. The *upper band* on the gels is dephosphorylated regulatory light chain; just below is the phosphorylated light chain. *17K* is the molecular weight 17,000 light chain, which migrates the most rapidly.

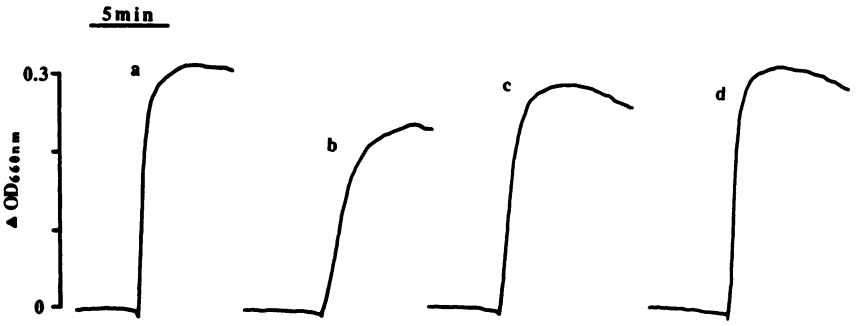

Fig. 5. Effect of *l*-T_4 on superprecipitation of actomyosin from bovine aorta smooth muscle. Superprecipitation was determined by measuring the increase in absorbance at 660 nm. ATP, 2 mM, was added at the *arrow* in the absence or presence of *l*-T_4. a, control; b, in the presence of 30 μM *l*-T_4; c, in the presence of 30 μM *l*-T_4 and 1 μg/ml CaM; d, in the presence of 1 μg/ml CaM.

TABLE 2

Effect of thyroid hormones on the two CaM-dependent protein kinases

The IC_{50} value was defined as the concentration of a drug required to produce a 50% inhibition of activation by CaM of MLCK or CaM kinase II. The values are means of two independent determinations, each performed in duplicate.

Inhibitor	IC_{50}	
	MLCK	CaM kinase II
	μM	
l-T_4	8.9 ± 2.0	22 ± 4
d-T_4	8.3 ± 1.6	44 ± 8.6
l-T_3	22 ± 4.0	76 ± 7.8
d-T_3	20 ± 3.3	47 ± 1.5

K^+ -stimulated arterial strips was not affected significantly by the doses of *l*-T_4 producing vascular relaxation.

On the other hand, we have already shown that W-7, a CaM antagonist, directly modulates the MLCK activity by competition for Ca^{2+}/CaM and produces vascular relaxation (5, 21, 22). Alternatively, we synthesized a direct inhibitor of MLCK, ML-9, and reported the importance of LC_{20} phosphorylation in platelet function (23) and in contractile response of vascular smooth muscle (8, 16). In the previous work (18), we showed that *l*-T_4 binds at or near the CaM binding domain of MLCK and inhibits CaM-induced activation of MLCK. In the present study, we examined the effect of *l*-T_4 on superprecipitation of actomyosin from bovine aorta in order to determine whether *l*-T_4 has an intracellular site of action. The data in Fig. 5 suggest that *l*-T_4 has inhibitory effects on Ca^{2+}/CaM-related regulatory mechanism(s) at the intracellular level. Although other possibilities cannot be excluded completely, we feel it is likely that *l*-T_4 inhibits vascular contraction via the inhibition of MLCK. W-7 inhibited the two Ca^{2+}/CaM-dependent protein kinases, MLCK (22, 24) and CaM kinase II,[2] with a similar concentration dependency, whereas *l*-T_4 was much more potent in its ability to inhibit MLCK than CaM kinase II (Table 2). All these results indicate that *l*-T_4 is neither a Ca^{2+} channel blocker nor a CaM antagonist but is a unique type of MLCK inhibitor that has inhibitory effects on vascular contraction.

In summary, we have demonstrated that thyroid hormones inhibit tension development, tension maintenance, and LC_{20} phosphorylation without significant inhibition of the influx of extracellular Ca^{2+} in vascular smooth muscle. Furthermore, *l*-T_4 was found to inhibit the superprecipitation of bovine aorta actomyosin. Although the discrepancy between the concentrations required for the inhibition of tension development and induction of relaxation of previously contracted vascular strips is considerable, and further studies are required to determine the mechanism of action of thyroid hormones, these thyroid hormones have an intracellular site of action, and the vasoinhibitory effects of *l*-T_4 may be at least in part accounted for by the inhibition of MLCK activity. These findings suggest the possibility that thyroid hormones may have a direct inhibitory effect on the actomyosin system and *l*-T_4 may be a pharmacological probe to investigate, as a selective MLCK inhibitor the function of MLCK *in vitro* and *in vivo*.

[2] Matsushima *et al.*, unpublished observations.

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