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Thyroid hormone affects both endothelial and vascular smooth muscle cells in rat arteries

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ABSTRACT

Hypothyroidism impairs endothelium-dependent dilatations, while hyperthyroidism augments the production of endothelial nitric oxide. Thus, experiments were designed to determine if thyroid hormone causes endothelium-dependent responses, or alleviates diabetic endothelial dysfunction. Isometric tension was measured in rings with or without endothelium of arteries from normal and diabetic Sprague-Dawley rats. Release of 6-keto prostaglandin $F_{1\alpha}$ and thromboxane B_2 were measured by enzyme linked immunosorbent assay and protein levels [endothelial nitric oxide synthase (eNOS), cyclooxygenases (COX)] by immunoblotting. Triiodothyronine (T_3) caused concentration-dependent (3×10^{-6}–3×10^{-5} M) relaxations in mesenteric (pEC_{50}, 4.96 ± 0.19) and femoral (pEC_{50}, 4.57 ± 0.35) arteries without endothelium. In femoral arteries of rats with diabetes, 5-methylamino-2-[[(2*S*,3*R*,5*R*,8*S*,9*S*)-3,5,9-trimethyl-2-(1-oxo-(1*H*-pyrrol-2- -yl)propan-2-yl)-1,7-dioxaspiro-(5,5)undecan-8-yl]methyl]benzooxazole-4-carboxylic acid (A23187, 3×10^{-7} to 10^{-6} M) caused partly endothelium-dependent contractions. After chronic T_3-treatment with (10 μg/kg/day; four weeks), the contractions to A23187 of preparations with and without endothelium were comparable, the thromboxane B_2-release was reduced (by $38.1\pm9.2\%$). The pEC_{50} of 9, 11-dideoxy-11α, 9α-epoxymethanoprostaglandin $F_{2\alpha}$ (U46619, TP-receptor agonist) was increased in T_3-treated diabetic rats compared with controls (8.53 ± 0.06 *vs* 7.94 ± 0.09). The protein expression of eNOS increased (by 228%) but that of COX-1 decreased (by 35%) after chronic T_3 treatment. In human umbilical vein endothelial cells incubated for one week with T_3 (10^{-10}–10^{-7} M) in the presence but not in the absence of interleukin-1β (1 ng/ml), the expression of eNOS was increased compared to control. In conclusion, thyroid hormone acutely relaxes mesenteric and femoral vascular smooth muscle, but given chronically reduces the release of endothelium-derived vasoconstrictor prostanoids while enhancing the responsiveness of TP receptors of vascular smooth muscle.

1. Introduction

The endothelium, a thin layer of cells lining the interior surface of blood vessels, can control local vascular tone. It does so by releasing endothelium-derived relaxing factors (Furchgott and Vanhoutte, 1989; Furchgott and Zawadzki, 1980), including nitric oxide (NO) and/or several other endothelium-derived hyperpolarizing substances (Félétou and Vanhoutte, 2007, 2006; Vanhoutte 2004). In addition, in particular in arteries of obese, diabetic or hypertensive animals, the release of endothelium-derived contracting factors [EDCFs], causing activation of the underlying smooth muscle cells (Tang and Vanhoutte, 2010; Vanhoutte, 2011; Wong and Vanhoutte, 2010c), contributes to endothelium-dependent changes in vascular diameter.

Several hormones can trigger endothelium-dependent responses. These include catecholamines acting on endothelial α2-adrenergic

Abbreviations: A23187, 5-methylamino-2-[[(2*S*,3*R*,5*R*,8*S*,9*S*)-3,5,9-trimethyl-2-(1-oxo-(1*H*-pyrrol-2-yl)propan-2-yl)-1,7-dioxaspiro-(5,5)undecan-8-yl]methyl] benzooxazole-4-carboxylic acid; COX, cyclooxygenase; EC, endothelial cell; EDCFs, endothelium-derived contracting factors; eNOS, endothelial nitric oxide synthase; HUVECs, human umbilical vein endothelial cells; L-NAME, Nω-nitro-L-arginine methyl ester; NO, nitric oxide; S18886, 3-[(6-amino-(4-chlorobenzensulphonyl)-2-methyl-5,6,7,8-tetrahydronapht]-1-yl)propionic acid; SD, Sprague-Dawley; SHR, spontaneously hypertensive rat; T_3, 3, 5, 3′-tri-iodothyronine; TP receptor, thromboxane prostanoid receptor; U46619, 9, 11-dideoxy-11α, 9α-epoxymethanoprostaglandin $F_{2\alpha}$

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receptors (Vanhoutte and Miller, 1989), vasopressin and oxytocin activating V1-vasopressinergic receptors (Katusic et al., 1986, 1984), and insulin (Liu et al., 2012a). Hormones, in particular estrogens, also chronically modulate endothelium-dependent responses (Chambliss and Shaul, 2002; Gisclard et al., 1988).

Hypothyroidism causes impaired endothelium-dependent dilatations (Taddei et al., 2003). Furthermore, diabetic patients have a higher prevalence of thyroid disorders compared to the normal population (Hage et al., 2011). Thyroid hormone is synthesized and stored in the follicular and the colloid cells of the thyroid gland and plays a role in differentiation, growth, and metabolism (Yen, 2001). The hormone also affects the cardiovascular system. Thus, hyperthyroidism results in increased heart rate and atrial fibrillation, while hypothyroidism causes opposite changes (Ichiki, 2010). Although thyroid hormone can cause vasodilatation (Carrillo-Sepúlveda et al., 2010; Ishikawa et al., 1989), its acute effect on endothelial cells is controversial, since both an absence of effect of 3, 5, 3′-tri-iodothyronine (T_3) on NO production (Ojamaa et al., 1996) and activation of endothelial NO synthase through the PI3K/Akt pathway (Hiroi et al., 2006) have been reported. Therefore, the present experiments were designed to determine in isolated arteries of normal rats whether or not the acute exposure to thyroid hormone causes or affects endothelium-dependent responses. Furthermore, clinical studies suggest impaired endothelium-dependent dilatations in hypothyroid patients (Lekakis et al., 1997) while hyperthyroidism causes excessive endothelial NO production (Napoli et al., 2001). Such an increased production of NO should reduce the occurrence of endothelium-dependent contractions (Tang et al., 2005a). Thus, the present study also investigated whether or not chronic treatment with thyroid hormone can alleviate the exaggerated EDCF-mediated responses that characterize the endothelial dysfunction resulting from type 1 diabetes (Shi et al., 2007a).

2. Materials and methods

All experimental protocols were approved by The University of Hong Kong Committee on the Use of Live Animals for Teaching and Research.

2.1. Experimental animals

The experiments were performed on isolated arteries of male Sprague Dawley (SD) rats. The rats used to test the direct effect of thyroid hormone were eight weeks old (250–350 g). To test the effect of the hormone on endothelial dysfunction, twelve weeks old rats (450–600 g) were made diabetic by the intraperitoneal administration of streptozotocin [30 mg/kg; dissolved in citric acid-trisodium citrate (0.2 mM) buffer (pH 4.0–4.5)] given once per day for three consecutive days (Shi et al., 2006). Seventy-two hours after the last injection, tail blood samples were obtained, and the glucose concentration was measured using a one-touch glucometer (LifeScan Inc., Milpitas, CA, USA). Induction of diabetes was considered successful when the fasting glucose level was higher than 16.6 mM. The diabetic rats were divided randomly into two groups, one receiving vehicle alone and the other a daily intraperitoneal injection of T_3 (10 μg/kg/day). T_3 was selected over thyroxine, because it mediates the effects of the latter *in vivo* and is more widely used in experimental animals (Carrillo-Sepúlveda et al., 2010; Dillmann, 1982; Hiroi et al., 2006; Szkudelski et al., 2003). Doses of 3 μg/kg (considered physiological) and 30 or 50 μg/kg (considered pharmacological) of T_3 have been administrated daily to diabetic rats by others investigators (Dillmann, 1982; Szkudelski et al., 2003). Since 3 μg/kg has no obvious metabolic effect, preliminary experiments were performed using 10 μg/kg, 50 μg/kg and 100 μg/kg of T_3, however, the latter two doses were severely toxic in diabetic rats, prompting the use of 10 μg/kg/day for further studies.

The rats were housed in the laboratory animal unit of The University of Hong Kong, and fed with normal chow. Water was provided *ad libitum*. The diabetic rats were studied four weeks after the last streptozotocin injection. On the day of the experiment, the non-fasting glucose level was measured again. After three h fasting, the rats were anesthetized with sodium pentobarbitone (70 mg/kg, intraperitoneally) and euthanized. Blood samples were collected from the inferior vena cava for measuring the T_3 level in serum.

2.2. Tissue preparation

The thoracic aorta, mesenteric arteries or femoral arteries were dissected free and placed in ice-cold modified Krebs–Ringer solution of the following composition (mM): NaCl 120, KCL 4.76, $CaCl_2$ 2.5, $MgSO_4$ 1.18, $NaHCO_3$ 25.0, NaH_2PO_4 1.18 and calcium disodium ethylenediaminetetraacetic acid 0.026, glucose 5.5 (control solution). The blood vessels were cut into rings (3–4 mm length in aorta, 1.5–2 mm length in mesenteric and femoral arteries). In some preparations, the endothelial cell layer was removed by the injection of 1 ml of Triton (0.5%, diluted in control solution) in the artery prior to cutting it into rings.

2.3. Isometric tension measurement

The preparations were suspended in organ chambers, which contained warmed (37 °C), aerated (95% O_2, 5% CO_2) control solution (5 ml). They were connected to a force transducer and a bio-signal acquisition system (PowerLab, ADInstruments, Sydney, Australia) to record isometric tension. The rings were stretched to an optimal tension (2.5 g in aorta, 2 g in mesenteric and 1 g in femoral arteries; determined in preliminary experiments; data not shown) and allowed to equilibrate for 90 min. They were then exposed twice to 60 mM KCL to obtain a reference contraction.

To study the acute vascular effects of T_3 in normal aorta, mesenteric or femoral arteries, U46619 (3×10^{-8} M) was added to the chamber and when a stable contraction level had been reached, cumulative concentrations (10^{-7} to 3×10^{-5} M) of T_3 were administered. In some experiments, the rings were first incubated with pharmacological inhibitors (Table 1). The concentrations of the inhibitors tested were selected from earlier work in the laboratory or from the literature (Table 1).

To examine whether or not the hormone has indirect effects, 10^{-7} M T_3 (a concentration that has no direct effect) was added to the organ chamber; after an incubation period of 30 min, cumulative concentrations of phenylephrine (10^{-9}–10^{-5} M) were given to quiescent rings or cumulative concentrations of acetylcholine (10^{-9}–10^{-6} M) were added during contractions to phenylephrine (10^{-6} M).

To study the effect of chronic treatment with thyroid hormone on endothelium-dependent relaxations, cumulative concentrations of acetylcholine (10^{-9}–10^{-6} M) were added during contractions to phenylephrine (10^{-6} M) to rings of the aorta and mesenteric arteries from both T_3 and vehicle treated diabetic rats.

To study the acute effect of thyroid hormone on endothelium-dependent contractions, rings of femoral arteries from diabetic rats were incubated with L-NAME (3×10^{-4} M, 30 min) plus T_3 (10^{-7} M), and exposed to cumulative concentrations of the calcium ionophore A23187 (10^{-8}–10^{-6} M).

To study the effect of chronic treatment with thyroid hormone on endothelium-dependent contractions, rings of femoral arteries from both T_3 and vehicle treated group were incubated with L-NAME (3×10^{-4} M, 30 min) or L-NAME (3×10^{-4} M, 30 min) plus indomethacin [cyclooxygenase (COX) inhibitor, 5×10^{-6} M, 30 min] or S18886 [thromboxane prostanoid (TP) receptor antagonist, 10^{-7} M,