A POSSIBLE ROLE FOR Ca^{2+} IN THYROID HORMONE-DEPENDENT OXYGEN CONSUMPTION IN SKELETAL MUSCLE OF THE RAT

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1. Introduction

The contribution of the energy cost of active Na^+–K^+ transport for the basal metabolic rate (BMR) [1–6] is the subject of controversy. The reported values vary from 5–50%. It is noteworthy that in studies making use of preparations in which organ integrity is preserved, low values are found [1,3,6], i.e. <15%. The importance of this question is illustrated by the fact that the energy spent to maintain Na^+ and K^+ gradients has been taken as the basis for theories explaining the thermogenic action of thyroid hormones, the evolution of endothermy, and non-shivering thermogenesis [4,5]. K^+-induced depolarization mobilizes intracellular Ca^{2+} [7,8], while dantrolene sodium interferes with it [9,10]. Here we show that K^+-induced depolarization stimulates the resting O_2 consumption in skeletal muscle increasingly in the direction hypothyroid → hyperthyroid. Dantrolene sodium decreases the resting O_2 consumption of skeletal muscle in euthyroid and hyperthyroid rats, the effect being largest in the hyperthyroid group. No effect was observed in the hypothyroid group. These data provide evidence that, at least in skeletal muscle, part of thyroid hormone-induced oxygen consumption is mediated by the alteration of Ca^{2+} fluxes in the cell.

2. Methods

Use was made of male rats, and all experiments were carried out in a perfused hind-limb system. The perfusion technique, described in [11], is basically derived from the method in [12]. This preparation is metabolically stable in the 15–90 min perfusion period. At the time of the perfusion experiments, the animals weighed 180–220 g. Hyperthyroidism was induced by daily subcutaneous injections of 15 μg T_3/100 g body wt over 10 days. Hypothyroidism was induced by one injection of 0.75 mCi ^{131}I given to animals which had been put on a low-iodine diet 2 weeks before. This treatment results in depletion of thyroid hormone within a week and a 50% decrease of oxygen consumption 3 weeks after the injection [13], the time at which the animals were used in these experiments.

The hind-limbs were perfused with recirculating semisynthetic medium consisting of Krebs-Ringer bicarbonate buffer (pH 7.4) supplemented with bovine erythrocytes (80 g/l), albumin (BSA) (0.001 M), 12 mM glucose, and 15 mM pyruvate. The medium was gassed with O_2 + CO_2 (95:5).

After perfusion had been started, the first 40 ml of the 120 ml perfusion medium was discarded and the remaining 80 ml was recirculated from that time (t = 0) at a flow rate of 0.4 ml . g muscle^{-1} . min^{-1}. The oxygen saturation was monitored with a PO_2 electrode type E 5047 (Copenhagen), and the oxygen consumption was calculated from the arteriovenous differences in O_2 content multiplied by the flow rate. Potassium was determined by flame photometry of samples of the perfusate supernatant. Dantrolene sodium was obtained from Norwich Benelux, Utrecht.

3. Results and discussion

Elevation of (K^+) in the medium to 20 mM resulted in an immediate sharp increase of the oxygen consumption in all groups (table 1). This increase was highest in the hyperthyroid group and lowest in the

Table 1
Effect of dantrolene sodium on oxygen uptake (μmol . g muscle^{-1} . min^{-1}) by rat hind-limbs in different thyroid states and perfused with medium containing 20 mM K^+

Condition	1. Basal (15–30 min)	2. K^+ at 20 mM (30–45 min)	3. K^+ at 20 mM + dantrolene (35 μM) (45–60 min)	Δ 2–1	Δ 3–1
Hypothyroid (n = 5)	0.35 ± 0.02[a]	0.73 ± 0.10[a]	0.37 ± 0.04[a]	0.39 ± 0.09[a]	0.02 ± 0.03
Euthyroid (n = 5)	0.58 ± 0.02	1.24 ± 0.08	0.55 ± 0.04	0.66 ± 0.08	−0.03 ± 0.02
Hyperthyroid (n = 5)	0.78 ± 0.08[a]	1.61 ± 0.10[a]	0.66 ± 0.08[a]	0.85 ± 0.07[a]	−0.12 ± 0.02[a]

[a] $P < 0.05$ *vs* euthyroid

Values (means ± SD) represent the oxygen uptake of perfused hind-limbs before (basal) and after successive addition of 20 mM K^+ and 20 mM K^+ + 35 μM dantrolene sodium to the medium. The intervals shown between parentheses indicate the time and duration of perfusion

hypothyroid group. The increase of oxygen consumption after K^+-induced depolarization appears to be related to Ca^{2+} release from the sarcoplasmic reticulum (SR) [7,8]. Probably, more Ca^{2+} is released from the SR by K^+-induced depolarization in the direction hypothyroid → hyperthyroid.

Next, dantrolene sodium, a muscle relaxant thought to interfere with the release of Ca^{2+} from the SR [9,10,14,15] was added. The K^+-induced oxygen-consumption increase was abolished in all groups. The inhibitory effect of dantrolene sodium was largest in the hyperthyroid group. Furthermore, the data in table 1 indicate that after the addition of dantrolene sodium there was actually a decrease of oxygen consumption below the base-line level in euthyroid and hyperthyroid rats that was not observed in the hypothyroid rats.

In a second experiment, dantrolene sodium (35 μM) was added to the perfusate after metabolic stabilization of the muscle preparation (t = 30 min) (fig.1). In hypothyroid rats no effect on the oxygen consumption was detected. Euthyroid and hyperthyroid rats showed a rapid decrease of oxygen consumption. The decrease was significantly greater ($P < 0.05$) at all time-points in the hyperthyroid group and reached a maximum between 60 and 90 min (euthyroid: 0.11 ± 0.03; hyperthyroid: 0.25 ± 0.08 μmol . g muscle^{-1} . min^{-1} (n = 6). Dantrolene sodium had no effect on K^+ release, which was very small (<0.05 μequiv . g muscle^{-1} . min^{-1}) and can be ascribed at least partially to a small degree of haemolysis occurring during the perfusion period.

Recently, high-affinity binding sites in sarcoplasmic vesicles of skeletal muscle have been identified for dantrolene sodium [16]. The possibility that these binding sites increase in the direction hypothyroid → hyperthyroid deserves further study.

Increased passive permeability of sarcoplasmic vesicles to Ca^{2+} has been found in the hyperthyroid rat heart [17]. Kinetic studies on Ca^{2+} efflux from

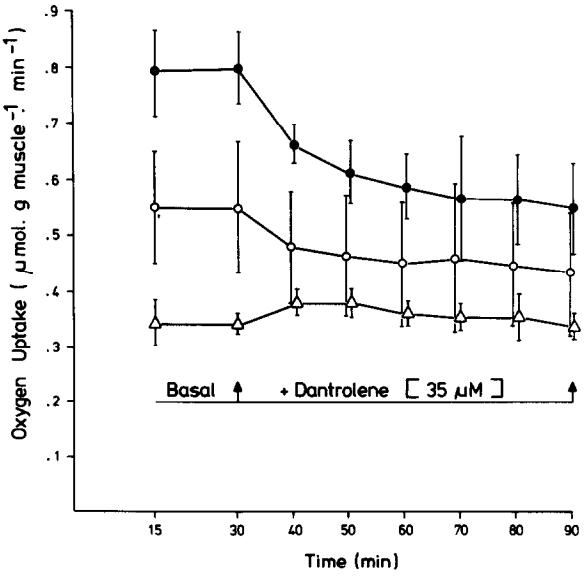

Fig.1. O_2 uptake (means ± SD) of perfused hind-limbs from hypothyroid (△) (4), euthyroid (○) (6), and hyperthyroid (●) (6) rats in the absence and presence of dantrolene sodium; no. expt in parentheses.

liver cells [18] and fat cells [19] of hypothyroid rats showed a diminished exchangeable organelle-bound Ca^{2+} pool. If the thyroid state alters Ca^{2+} fluxes from the SR, this could cause small changes of cytosolic $[Ca^{2+}]$ which might have a profound influence on mitochondrial oxidative metabolism [20].

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