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Integrin participates in the effect of thyroxine on plasma membrane in immature rat testis

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ABSTRACT

Background: The secretory activity of Sertoli cells (SC) is dependent on ion channel functions and protein synthesis and is critical to ongoing spermatogenesis. The aim of this study was to investigate the mechanism of action associated with a non-metabolizable amino acid [^{14}C]-MeAIB (α-(methyl-amino)isobutyric acid) accumulation stimulated by T_4 and the role of the integrin receptor in this event, and also to clarify whether the T_4 effect on MeAIB accumulation and on Ca^{2+} influx culminates in cell secretion.
Methods: We have studied the rapid and plasma membrane initiated effects of T_4 by using $^{45}Ca^{2+}$ uptake and [^{45}C]-MeAIB accumulation assays, respectively. Thymidine incorporation into DNA was used to monitor nuclear activity and quinacrine to analyze the secretory activity on SC.
Results: The stimulation of MeAIB accumulation by T_4 appears to be mediated by the integrin receptor in the plasma membrane since tetrac and RGD peptide were able to nullify the effect of this hormone. In addition, T_4 increases extracellular Ca^{2+} uptake and Ca^{2+} from intracellular stocks to enhance nuclear activity, but this genomic action seems not to influence SC secretion mediated by T_4. Also, the cytoskeleton and ClC-3 chloride channel contribute to the membrane-associated responses of SC.
Conclusions: T_4 integrin receptor activation ultimately determines the plasma membrane responses on amino acid transport in SC, but it is not involved in calcium influx, cell secretion or the nuclear effect of the hormone.
General significance: The integrin receptor activation by T_4 may take a role in plasma membrane processes involved in the male reproductive system.

1. Introduction

Thyroid hormones (TH) exert a broad range of effects on cell development, growth, differentiation and metabolism, but not all of these actions are related to nuclear transcription [1]. The pro-hormone thyroxine, 3,5,3′,5′-L-tetraiodothyronine (T_4), is synthesized only by the thyroid gland whereas 3,5,3′-L-triiodothyronine (T_3) and 3,3′,5′-triiodothyronine (rT_3) are produced by both the thyroid and by deiodination of T_4 at extrathyroidal sites. The circulating T_3 is generated by pre-receptor ligand metabolism resulting from the activity of the iodothyronine deiodinase enzymes D1 and D2, which convert T_4 to T_3, via 5′ monodeiodination. Diiodothyronine, or T_2, can exist in three forms and oxidative deamination and decarboxylation of T_4 result in the formation of tetraiodothyroacetic acid (tetrac). Triiodothyroacetic acid (triac) can be formed from T_3 in a similar way to tetrac [2,3].

In the testis, Sertoli cells (SC) and the specialized junctions between them and the neighboring germ cells create a sophisticated microenvironment providing all the nutrients and growth factors required for the full development of spermatogenetic cells, and the mechanisms that regulate SC metabolism are central to the maintenance of spermatogenesis and male fertility [4,5]. SC is the major source of lactate in the testis, and produces this metabolite primarily from glucose. Lactate is the preferred energy substrate for spermatocytes and spermatids [6,7], leading to the conclusion that changes in carbohydrate metabolism of SC may result in a compromised spermatogenesis. Furthermore, acetate and acetate metabolism have a probable role in producing sub-products essential to maintain a high rate of lipids synthesis in the developing germ cells [8]. Additionally, Alves et al. [8] showed that acetate metabolism is under 5α-dihydrotestosterone and 17β-estradiol regulation and insulin-deprivation dramatically changes human SC metabolism by completely suppressing acetate production and exports (for review see [9]). Although the action of TH on Sertoli cells and the role of

Abbreviations: TH, thyroid hormones; T_4, 3,5,3′,5′-L-tetraiodothyronine; T_3, 3,5,3′-L-triiodothyronine; rT_3, 3,3′,5′-triiodothyronine; tetrac, tetraiodothyroacetic acid; RGD, Arg-Gly-Asp; MeAIB, methylaminoisobutyric acid; SC, Sertoli cells

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this nurse cells to supply the physical and nutritional support for germ cells be well described, the mechanism of action of thyroxine in the testis is not completely understood [10,11].

Several reports have discussed about genomic and nongenomic signal transducing triggered by TH in the testis [11,12]. Particularly in the testis, our group has showed nongenomic effect of TH using different protocols [13–16] but the site of action to thyroxine at plasma membrane needs to be elucidated.

TH receptors are highly expressed in neonatal Sertoli cells, indicating that the developing of these cells and testis may be important TH targets. Biochemical effects of TH demonstrate that the SC is the main direct target in the testis for TH, and that the prepuberal period is the temporal frame for its action [17,18]. Although the action of TH on SC function has received much attention since the finding of functional T_3 receptors in immature rat testis, being almost exclusively located in SC [17,19], the precise function of TH in the testis is unsatisfactorily defined.

The actions of TH in target tissues are predominantly mediated by specific nuclear receptors capable of binding to regulatory regions of target genes and modifying their expression [20]. Nongenomic actions of TH are widely acknowledged but the specific cellular target is quite difficult to characterize since it can be initiated in the plasma membrane, cytoplasm, cytoskeleton or sub-cellular organelles [10,21,22]. Nongenomic responses do not require the production of new protein(s), occur in the extranuclear milieu of the cell and can culminate in the regulation of genes that do not contain a TH response-element [21,23]. These actions are regulated by specific agonists and antagonists, have a short latency and are not affected by transcription and translation inhibitors.

In particular, in the case of T_4, two reports from our group show that the stimulatory effect of T_4 on amino acid accumulation (a specific plasma membrane transport system), is independent of active protein synthesis. Moreover, it was demonstrated that the immediate hyperpolarizing effect of T_4 on SC is influenced by Ca^{2+}-activated K^+ channels [11]. Following these findings, Menegaz et al. [16] demonstrated that the stimulatory effects of T_4 on Ca^{2+} uptake and on amino acid accumulation, both events initiated in the plasma membrane which strongly characterizes a nongenomic effect, are mediated by T_4 interaction with the SC plasma membrane and the opening of ATP-dependent K^+, Ca^{2+}-dependent K^+ and Cl^- channels hyperpolarizing the cells. This hyperpolarization induces the opening of voltage-dependent Ca^{2+} channels, Ca^{2+} influxes and "depolarization" which trigger Na^+-amino acid co-transport. The local Ca^{2+} transient activates protein kinase C (PKC) that may regulate plasma membrane ionic channel activities and/or promote intracellular "cross-talk" to ultimately modulate gene transcription or keep the ongoing secretory activity. Based on the possibility that integrin $\alpha_v\beta_3$ mediates TH to induce angiogenesis [24], the current experiments were designed to investigate the hypothesis that T_4 interacts with the integrin receptor to mediate rapid responses in SC.

2. Materials and methods

2.1. Materials

L-thyroxine (T_4), 3,5,3′-triiodo-L-thyronine (T_3), 3,3′,5′-triiodothyronine (reverse T_3, rT_3), tetraiodothyroacetic acid (tetrac), Arg-Gly-Asp (RGD), ethyleneglycol-O-OV-bis(2-aminoethyl)- NV,N,NV,NV-tetraacetic acid (EGTA), 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis (acetoxymethyl ester) (BAPTA-AM) 4, 4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), verapamil, colchicine and quinacrine were purchased from Sigma Aldrich Chemical Company, St. Louis, MO, USA. α-[1-^{14}C] methylaminoisobutyric acid ([^{14}C] MeAIB) (sp.act. 1.85 GBq/mmol), thymidine [methyl-^{14}C] (sp. act. 1.7464 GBq/mmol), [^{45}Ca]$CaCl_2$ (sp. act. 321 KBq/mg Ca^{2+}), and Optiphase Hisafe III biodegradable liquid scintillation fluid were purchased from PerkinElmer (Boston, USA). All other chemicals were of analytical grade.

2.2. Animals

Wistar rats bred in our animal house and maintained in an air-conditioned room (21 °C) with controlled lighting (12 h/12 h light/dark cycle) were used in this study. The suckling rats were kept with their mothers until sacrifice by cervical dislocation. Pelleted food (Nuvital, Nuvilab CR1, Curitiba, PR, Brazil) and tap water were available *ad libitum*. All the animals were carefully monitored and maintained in accordance with the ethical recommendations of the Brazilian Veterinary Medicine Council and the Brazilian College of Animal Experimentation (Protocol CEUA/PP00418).

2.3. Amino acid accumulation measurements

For the amino acid accumulation experiments one gonad (alternately left and right) of 11-day-old rats was used as the experimental tissue and the contralateral one was used as the control. The testes were weighed, decapsulated and pre-incubated in Krebs Ringer bicarbonate (KRb) buffer (122 mM NaCl; 3 mM KCl; 1.2 mM $MgSO_4$; 1.3 mM $CaCl_2$; 0.4 mM KH_2PO_4; 25 mM $NaHCO_3$ and 5 mM glucose) for 30 min in a Dubnoff metabolic incubator at 34 °C, pH 7.4 and gassed with O_2:CO_2 (95:5; v/v). T_4 (10^{-9} M), rT_3 (10^{-6} to 10^{-10} M), tetrac (10^{-9} M), RGD (5×10^{-7} M), colchicine (10^{-6} M) and DIDS (200 μM) were added to the pre-incubation and incubation media and the concentrations used in these assays were selected based in our previous studies [11,25] and those of other authors [26]. T_4 was dissolved in 0.025 M NaOH-saline. This solution was further diluted to the final concentrations in KRb. The buffer was bubbled with 95% O_2–5% CO_2 up to pH 7.4. The gonads were then incubated in fresh KRb buffer for 60 min. [^{14}C] MeAIB (3.7 kBq/mL) was added to each sample during the incubation period [13]. After incubation the testes were placed in screw cap tubes containing 1 mL of distilled water. They were frozen at −20 °C in a freezer and afterwards boiled for 5 min; 25 μL aliquots of tissue and external medium were placed in scintillation fluid and counted in a Beckman beta liquid scintillation spectrometer (model LS 6500; Fullerton, California, USA) for radioactivity measurements. The results were expressed as the tissue/medium (T/M) ratio: cpm/mL tissue fluid per cpm/mL incubation medium [11,13].

2.4. $^{45}Ca^{2+}$ uptake

One gonad (alternately left and right) from 11-day-old rats was used as experimental tissue and the contralateral one was used as the control. The testes were decapsulated and pre-incubated in KRb buffer for 15 min in a Dubnoff metabolic incubator at 34 °C, pH 7.4 and gassed with O_2:CO_2 (95:5; v/v). The testes were then transferred to another series of wells containing fresh KRb with 0.1 μCi/mL $^{45}Ca^{2+}$ and left for 60 min. Verapamil (10^{-4} M), RGD (5×10^{-7} M) or tetrac (10^{-9} M) was added during the last 15 min before the hormone addition and maintained during the whole incubation period. Finally, T_4 was added to these $^{45}Ca^{2+}$ solutions and the tissues were incubated with 10^{-9} M T_4 with/without verapamil, RGD or Tetrac for 60 s [16].

Extracellular $^{45}Ca^{2+}$ from the testis was thoroughly washed off in 127.5 mM NaCl, 4.6 mM KCl, 1.2 mM $MgSO_4$, 10 mM HEPES, 11 mM glucose, and 10 mM $LaCl_3$, at pH 7.4 (30 min in washing solution). The presence of La^{3+} during the washing stage was found to be essential to prevent release of the intracellular $^{45}Ca^{2+}$ [27]. After La^{3+} tissue washing, testes were homogenized with 0.5 M NaOH solution; 50 μL aliquots of tissue medium were placed in scintillation fluid for counting in a Beckman coulter beta liquid scintillation spectrometer (model LS 6500; Fullerton, California, USA), and 5 μL aliquots were used for total protein quantification by the Lowry method [28]. The results were expressed as pmol $^{45}Ca^{2+}$/μg of protein [16].