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INTRINSIC EXCITABILITY IS ALTERED BY HYPOTHYROIDISM IN THE DEVELOPING HIPPOCAMPAL CA1 PYRAMIDAL CELLS

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Abstract—Thyroid hormone plays an essential role in brain development, so its deficiency during a critical developmental period has been associated with profound neurological deficits, including irreversible mental retardation. Despite the importance of the disorder, the cellular mechanisms underlying these deficits remain largely unexplored. The aim of this study was to examine the effects of the absence of thyroid hormone on the postnatal development of membrane excitability of CA1 hippocampal pyramidal cells. Current clamp recordings in the whole cell patch clamp configuration showed that the action potential of cells from hypothyroid animals presented shorter width, slower depolarization, and faster repolarization rates compared with controls both in early postnatal and pre-weanling ages. Additionally, thyroid hormone deficiency reduced the intrinsic membrane excitability as measured by the reduced number of evoked action potentials for a given depolarizing slope and by the more depolarized firing threshold observed in hypothyroid animals. Then we analyzed the fast-repolarizing A- and D-type potassium currents, as they constitute one of the major factors underlying intrinsic membrane excitability. Hypothyroid rats showed increased A-current density and a reduced isolated I_D-like current, accompanied by parallel changes in the expression of the channels responsible for these currents in the CA1 region: Kv4.2, Kv4.3, and Kv1.2. Therefore, we suggest that the increased A-current density, subsequent to an increment in its channel expression, together with the decrease of Na^+-currents, might help explain the functional alterations in the neuronal discharge, in the firing threshold, and in the action potential repolarization of hypothyroid pyramidal neurons.

Key words: A-type potassium current, D-type potassium current, Kv4, Kv1.2, neuronal discharge, action potential.

Thyroid hormone is essential in brain development (Oppenheimer and Schwartz, 1997), its deficiency during a critical developmental period being associated with irreversible mental retardation and profound neurological deficits, including deafness and motor disorders (DeLong, 1989). Besides these alterations in humans, experimental hypothyroidism in developing rats leads to impaired learning (Schwartz, 1983; Akaike et al., 1991; Hashimoto et al., 2001), retarded locomotor ability (Hashimoto et al., 2001), hyperactivity (Akaike et al., 1991; Goldey et al., 1995), and abnormal brain development, with diminished interneuronal connectivity (Schwartz, 1983; Rami et al., 1986a,b). However, the precise cellular bases of these functional impairments are unknown.

Cognitive impairment often reflects compromised neuronal communication, which can result from a change in the intrinsic membrane properties that affect the efficiency with which synaptic input can be transformed into action potentials. In hippocampal CA1 pyramidal neurons, the change of intrinsic membrane properties that underlie intrinsic excitability relates to learning ability (Moyer et al., 1996; Thompson et al., 1996). In a previous study (Sánchez-Alonso et al., 2010), we found that the congenital absence of thyroid hormone alters the neuronal firing behavior during the early postnatal development in rat. Furthermore, our results supported that the changes in neuronal discharge are related to a decline in the low-threshold Ca^{2+} current (I_T). Thyroid deficiency also caused alterations in the firing threshold and the action potential (AP) repolarization of CA1 pyramidal neurons. Several potential mechanisms might explain these differences. On the one hand, the depolarized firing threshold observed in hypothyroid animals might be because of a lower density of voltage-dependent sodium channels, as the firing threshold depends largely on these channels (Hodgkin and Huxley, 1952; Carter and Bean, 2009). In agreement with this hypothesis, it has recently been shown that the lack of thyroid hormone in primary cultures of the hippocampus results in smaller sodium currents (I_{Na}) (Hoffmann and Dietzel, 2004). On the other hand, potassium currents are especially important for the regulation of neuronal excitability because they repolarize neurons in response to depolarizing events and help to stabilize the membrane potential below the firing threshold. The differences in AP width and fast repolarization rates might be because of changes in the fast repolarizing potassium currents such as the A- or D-type (Kim et al., 2005; Storm, 1987; Sah, 1996; Mitterdorfer and Bean, 2002). The channels underlying these currents are also active at subthreshold membrane potentials (Kim et al., 2005; Mitterdorfer and Bean, 2002), and thus an increase in these currents might also contribute to the differences observed in the firing threshold.

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Abbreviations: ACSF, artificial cerebrospinal fluid; AP, action potential; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; EGTA, ethylene glycol tetraacetic acid; G, conductance; I_A, A-type potassium current; I_D, D-type potassium current; I_{Na}, sodium current; I_T, T-type calcium current; MMI, methylmercaptoimidazole; PBS, phosphate-buffered saline; PTX, picrotoxin; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TEA, tetraethyl-ammonium; TTX, tetrodotoxin; TX, hypothyroid rats; TX+T3, hypothyroid rats injected with T3; T3, 3,3',5 triiodo-L-thyronine; τ_{fast}, fast time constant; τ_{slow}, slow time constant; 4-AP, 4-aminopyridine.

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In the present study, we seek to clarify the effects that the absence of thyroid hormone exerts on the postnatal development of the excitability of CA1 hippocampal pyramidal cells. For this, we focus on the first postnatal weeks, analyzing the alterations in the potassium currents involved in spike repolarization, as well as the alterations in the expression of their most representative channels. Specifically, we examine the changes of the two main types of low-voltage activated outward currents expressed in hippocampal CA1 cells during early postnatal development: A- and D-type currents.

EXPERIMENTAL PROCEDURES

Animals and drug administration

All experimental procedures described below followed the guidelines of the European Union (86/609/EEC) and Spanish legislation for the use and care of laboratory animals (BOE 65/8509-12, 1988). The experimental procedures included measures to reduce the number of animals used and minimize their suffering. Female Wistar rats were mated and the day of appearance of the vaginal plug was considered to be day 0 of fetal age. Fetal and neonatal hypothyroidism was induced as previously described (Lorenzo et al., 2002; Vara et al., 2002; Oh-Nishi et al., 2005; Giné et al., 2010). Dams were treated with 0.02% methylmercaptoimidazole (MMI) in the drinking water from day 9 of gestation and continued until the experiments were performed. This protocol provides a good hypothyroidism model for developing rats, as it results in low levels of circulating thyroid hormones (Lorenzo et al., 2002; Giné et al., 2010) and avoids surgery in neonatal animals. The decreased growth rate associated to the hypothyroidism was (a) P9–12 group, controls 21.64±0.34 g (n=89) vs. hypothyroid 16.76±0.37 g (n=86), $P<0.001$ and (b) P16–19 group, controls 36.64±0.47 g (n=115) vs. hypothyroid 26.68±0.5 g (n=101), $P<0.001$. For the thyroid hormone treated group (n=58), hypothyroid neonates were intraperitoneally injected daily with 3,3',5 triiodo-L-thyronine (T3; 20 μg of T3/100 g of body weight), starting 72 h before sacrificing/slicing. The corresponding controls received an equivalent volume of physiological saline.

Slice preparation

Rats were decapitated and the hippocampi quickly dissected in cold Krebs buffer. Slices were placed in a humidified holding chamber for at least 1 h (27–29 °C) in the same artificial cerebrospinal fluid (ACSF) used for recording. A single slice was transferred to the recording chamber and submerged in a continuously superfusing solution saturated with 95% O_2 5% CO_2. The composition of the ACSF was (in mM): 120 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 26.2 $NaHCO_3$, and 11 glucose, at pH 7.4 when equilibrated with 95% O_2 5% CO_2. Experiments were performed at 27–29 °C in order to ensure good clamping conditions.

Whole-cell patch-clamp recordings in hippocampal slices

"Blind" whole-cell patch-clamp recordings (Blanton et al., 1989; Coleman and Miller, 1989) were made from CA1 pyramidal cells. These experiments were performed with electrodes filled with (in mM): 125 K gluconate, 8 NaCl, 10 HEPES, 3 Tris–ATP, 0.3 GTP, 2 $MgCl_2$; 20 phosphocreatine, 0.5 $CaCl_2$, 10 EGTA, and 50 U/ml creatine kinase were included in the electrode. Patch pipettes filled with these solutions (Clark Electromedical, Reading, UK) had resistances of 5–10 MΩ. The glutamate receptor antagonists 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX; 10 μM) to block fast excitatory postsynaptic potentials, and the $GABA_A$ receptor antagonist picrotoxin (PTX; 100 μM) to block fast inhibitory postsynaptic potentials, were included in the ACSF. A peristaltic pump (Gilson, Villiers le Bel, France) was used to circulate the solution (1.5–2 ml/min) through the recording chamber, keeping the flow rate constant and avoiding any flow artifacts. Pipettes were pulled from borosilicate glass capillaries on a Flaming-Brown micropipette puller (Sutter Instruments, Novato, CA, USA). Current-clamp recordings were acquired with an Axoclamp 2A amplifier and Digidata 1322 interface (Molecular Devices, Union City, CA, USA). The pyramidal cells included in this study had stable resting potentials of at least −60 mV and exhibited an AP with peak voltage greater than 0 mV. Each cell was recorded briefly in current-clamp mode at −60 mV to assess basal and active membrane potential characteristics. Input resistance was calculated in some cells from the steady-state voltage measured in response to a −20 pA current pulse (300 ms duration). Series resistance was compensated for current-clamp recordings.

Acutely dissociated neurons

Potassium currents were recorded in the whole-cell configuration using the method described in detail by Kay and Wong (1986). In brief, transverse hippocampal slices were cut (500 μm thick) and incubated for at least 1 h. Five slices were removed from the holding chamber as required, and the CA1 region was dissected and cut into 0.5 mm^3 cubes, which were incubated at 27–29 °C in a 95% O_2 5% CO_2 saturated saline (in mM): 105 NaCl, 5 KCl, 1 $CaCl_2$, 1.3 $MgCl_2$, 25 glucose, and 10 HEPES, with 2 mg/ml protease (Sigma type XIV) for 15 min. Cubes were rinsed in the same saline solution without protease. Cells were dissociated by gently triturating the tissue through a series of fire-polished Pasteur pipettes and were plated onto poly-L-lysine-coated cover slips in a perfusion chamber (flow 1.3–1.6 ml/min) mounted onto a fixed-stage microscope. The composition of the solution was as follows (in mM): 105 NaCl, 2.5 KCl, 2 $CaCl_2$, 1.3 $MgCl_2$, 26.2 $NaHCO_3$, 3 $CoCl_2$, 0.0005 tetrodotoxin (TTX), 11 glucose, pH 7.4 when equilibrated with 95% O_2 5% CO_2. Patch pipettes were filled with a solution containing the following (in mM): 80 KCl, 10 EGTA, 10 HEPES, 4 Tris–ATP, 0.3 GTP, 4.5 $MgCl_2$, 14 phosphocreatine, and 40 sucrose (adjusted to pH 7.2 with KOH) and attached to cells of pyramidal shape by mild suction. Patch pipettes filled with these solutions (Clark Electromedical, Reading, UK) had resistances of 3–7 MΩ. Following seal formation (>2 GΩ) whole-cell recordings lasting >40 min were achieved by rupturing the neuronal membrane with further negative pressure. Recorded cells had a uniformly bright appearance and capacitances higher than 10 pF with a truncated dendritic arbor because of the dissociation process. Dissociated control cells presented a capacitance of 17.82±0.49 pF at P9–12 (n=78) and 17.50±0.49 pF at P16–19 (n=111), whereas hypothyroid cells presented statistically significant less capacitance than did controls ($P<0.01$): 15.63±0.45 pF at P9–12 (n=88) and 15.53±0.46 pF at P16–19 (n=90). This difference is because of the soma size (P9–12: 260.54±6.00 μm^2 at control vs. 223.85±6.19 μm^2 at hypothyroid rats (TX); P16–19: 262.03±7.66 μm^2 at control vs. 226.91±6.10 μm^2 at TX; $P<0.001$ at both ages), whereas it is not because of the dendritic size (P9–12: 176.35±7.40 μm^2 at control vs. 161.79±6.46 μm^2 at TX; P16–19: 169.22±6.18 μm^2 at control vs. 176.62±7.29 μm^2 at TX; with no significant difference at any age). Voltage-clamp recordings were acquired with an Axopatch-200B amplifier. Series resistances and membrane capacitances were partially compensated for 80%, and off-line filtering was not performed.

Electrophysiological data analysis

Action potential parameters were analyzed for the first AP induced by low-intensity (50–250 pA) long pulses (1 s) and high-intensity (400–900 pA) short pulses (5 ms) of current injection in CA1 pyramidal neurons recorded under current-clamp mode. The AP