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Modulatory Effects Of Polyphenol R-1 On Calcium Transport And Synaptosomal Function In Experimental Hypothyroidism

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Abstract: The present study investigated the corrective effect of the polyphenol R-1 on neuronal calcium transport and synaptosomal function in rats with experimental hypothyroidism. Hypothyroidism was induced in adult male rats by oral administration of mercazolil (2.5 mg/100 g) for 21 days. Model validation was confirmed through behavioral (Open Field Test), biochemical, and hormonal analyses. Hypothyroid rats exhibited decreased triiodothyronine (T3) and thyroxine (T4) levels, elevated thyrotropin (TSH), impaired biochemical parameters in brain homogenates, and a marked reduction in intracellular calcium concentration in brain synaptosomes. Treatment with polyphenol R-1 (50 mg/kg, orally, 14 days) significantly corrected behavioral hypoactivity, normalized serum hormone profiles, restored biochemical markers, and increased synaptosomal calcium levels toward control values. Mechanistically, R-1 modulated the activity of NMDA receptors and voltage-gated calcium channels, stabilizing calcium homeostasis and synaptic transmission. These findings demonstrate that polyphenol R-1 exerts neuroprotective and calcium-regulatory effects in hypothyroid-induced neuronal dysfunction, suggesting its potential as a therapeutic agent for thyroid-related neuropathologies.

Keywords: Hypothyroidism, Polyphenol R-1, NMDA receptor, Calcium homeostasis, Synaptosome, Neuroprotection.

Introduction: Hypothyroidism, characterized by insufficient production of thyroid hormones, induces widespread metabolic and neurophysiological disturbances. Thyroid hormones (T3 and T4) are crucial regulators of neuronal differentiation, ion channel expression, neurotransmitter metabolism, and mitochondrial bioenergetics [1,2]. Their deficiency disrupts neuronal calcium homeostasis by downregulating NMDA receptors, voltage-gated calcium (VGCC) and sodium (VGSC) channels, and ATP-dependent calcium pumps, leading to impaired neurotransmission and cognitive deficits [3–6].

Experimental hypothyroidism in rodents, induced by the antithyroid agent mercazolil (methimazole), is an established model for studying endocrine and neurochemical dysfunctions [7]. Our previous findings confirmed that mercazolil-treated rats display pronounced hypothyroid symptoms, decreased locomotor activity, altered biochemical parameters, and significantly reduced intracellular calcium levels in brain synaptosomes.

Polyphenols are known modulators of oxidative stress and ion channel activity [8,9]. Based on preliminary data, the novel polyphenol R-1 interacts with NMDA receptor regulatory sites and modulates calcium transport through synaptosomal membranes. This study aimed to evaluate the corrective effects of polyphenol R-1 on synaptosomal calcium dynamics, receptor function, and biochemical parameters in mercazolil-induced hypothyroidism.

METHODS

Animals and Experimental Design

Adult male outbred rats (220 ± 10 g) were maintained under standard vivarium conditions (22 ± 2 °C, 12-h light/dark cycle) with free access to food and water. Hypothyroidism was induced via oral mercazolil administration (2.5 mg/100 g/day) for 21 days using a gastric tube [7]. After model verification, rats were divided into three groups (n = 8):

1. Control group: intact animals;
2. Hypothyroid group: mercazolil-treated;
3. R-1-treated hypothyroid group: mercazolil + polyphenol R-1 (50 mg/kg, orally, 14 days).

All procedures followed institutional and international ethical standards for laboratory animal care.

Behavioral Testing

The Open Field Test was performed to assess locomotor and exploratory activity before and after treatment. Parameters such as horizontal and vertical movements, grooming, burrowing, and bolus defecation were recorded and analyzed using the McGraw scale.

Hormonal and Biochemical Analysis

Serum levels of T3, T4, and TSH were quantified using the HumaReader HS system. Brain homogenates were assayed for glucose, triglycerides, total protein, cholesterol, AST, and ALT using standard biochemical kits.

Isolation of Brain Synaptosomes

Synaptosomes were isolated from brain tissue by differential centrifugation according to Cotman's method [10]. Homogenization was performed in 0.32 M sucrose/0.01 M Tris-HCl/0.5 mM EDTA buffer (pH 7.4) at 4 °C. The suspension was centrifuged at 4500 rpm (10 min), followed by 14 000 rpm (20 min), and resuspended in Krebs-Ringer medium.

Measurement of Intracellular Calcium ($[Ca^{2+}]_i$)

Synaptosomal $[Ca^{2+}]_{in}$ was measured using the Fluo-4 AM fluorescent probe (5 μM) [11,12]. Fluorescence was excited at 488 nm and recorded at 506 nm using a USB-2000 spectrofluorimeter (Ocean Optics, USA). Calcium concentrations were calculated using the Grynkiewicz equation, with EGTA (1 mM) providing Fmin and $CaCl_2$ (2 mM) Fmax. Data were expressed as mean ± SEM; statistical significance was assessed by Student's t-test ($p < 0.05$).

RESULTS

Behavioral Findings

Mercazolil-treated rats exhibited typical hypothyroid symptoms: lethargy, poor appetite, constipation, and partial alopecia. Open field testing showed significant reductions ($p < 0.01$) in horizontal and vertical activity, grooming, and exploratory behavior compared with controls. Treatment with polyphenol R-1 markedly improved locomotor and exploratory indices, approaching control values, indicating restored CNS activity (Figure 1).

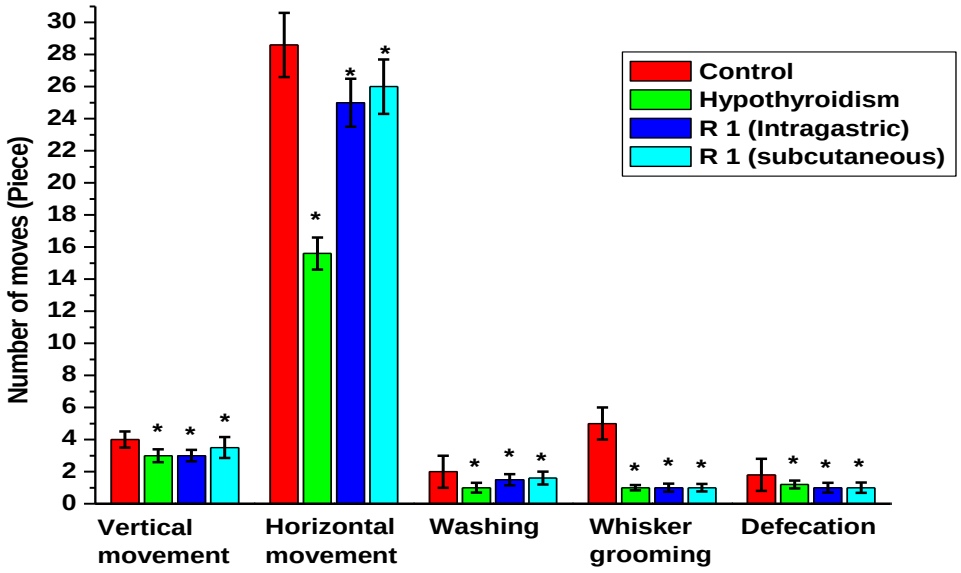

Figure 1. Assessment of cognitive behavior in the open field test for 3 minutes. (*p<0.05; n=6).

Hormonal and Biochemical Changes

As shown in Table 1, hypothyroid rats exhibited decreased serum T3 and T4 and elevated TSH, confirming hypothyroidism. R-1 treatment normalized T3/T4 levels and partially reduced TSH concentrations. Biochemical assays of brain homogenates revealed improved glucose, triglyceride, and protein levels after R-1 administration, along with decreased AST and ALT activities, suggesting hepatoneuronal recovery.

Synaptosomal Calcium Concentration

Fluo-4 AM fluorescence analysis showed a 35–40% reduction in $[Ca^{2+}]_{in}$ synaptosomes from hypothyroid rats compared with controls ($p < 0.01$). After polyphenol R-1 treatment, calcium levels significantly increased ($p < 0.05$), indicating restoration of Ca^{2+} transport capacity (Figure 2).

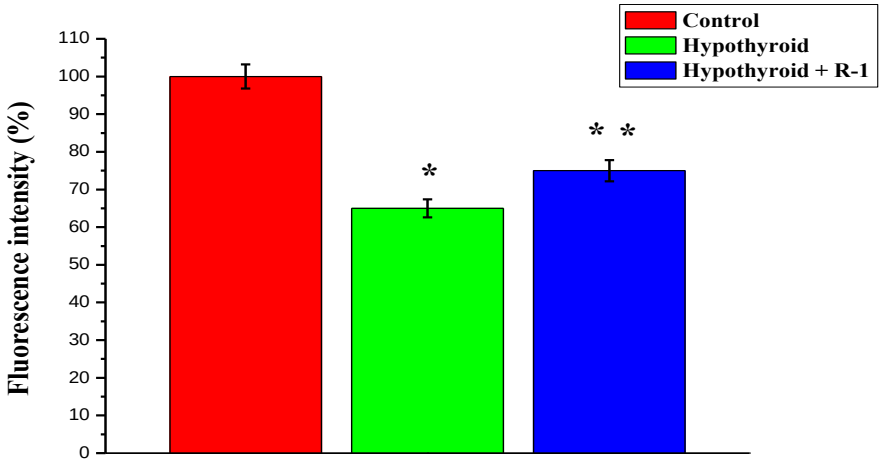

Figure 2. Effect of polyphenol R-1 on $[Ca^{2+}]_{in}$ levels in brain synaptosomes in hypothyroid rats. (*p<0.05; **p<0.01; n=6).

This normalization is likely mediated by enhanced NMDA receptor activation and re-established voltage-gated calcium channel function.

DISCUSSION

Hypothyroidism is known to impair neuronal excitability by decreasing expression and phosphorylation of NMDA receptor subunits and L-type VGCCs [13,14]. Reduced T3 levels downregulate the transcription of genes encoding NR1, NR2B, and Cav1.2 channels, resulting in diminished calcium influx and weakened synaptic transmission [15-18]. Our findings confirm this mechanism: mercazolil-induced hypothyroidism reduced synaptosomal $[Ca^{2+}]_{in}$, likely reflecting compromised receptor/channel function and mitochondrial Ca^{2+} buffering.

Polyphenol R-1 exerted a corrective effect by restoring synaptosomal Ca^{2+} levels, improving hormone profiles, and normalizing behavior. The underlying mechanisms may include:

Polyphenol R-1 interacts with glutamate-binding sites, preventing excessive receptor desensitization while maintaining Ca^{2+} conductance. By stabilizing membrane potential and redox balance, R-1 reactivates dihydropyridine-sensitive calcium channels (L-type). Polyphenol R-1 likely preserves mitochondrial potential, enhancing ATP-dependent Ca^{2+} pumps (PMCA/SERCA) and calcium homeostasis. Improved dopaminergic and serotonergic signaling explains behavioral normalization. Thus, R-1 displays neuroprotective, antioxidant, and calcium-modulatory actions that collectively reverse hypothyroid-induced synaptic dysfunction.

CONCLUSION

The mercazolil-induced hypothyroid rat model effectively reproduces the biochemical and behavioral features of hypothyroidism, including decreased synaptosomal calcium and impaired neural activity. Polyphenol R-1 demonstrates a corrective effect by restoring thyroid hormone balance, improving biochemical indices, normalizing synaptosomal calcium levels, and enhancing cognitive-motor function. These results indicate that polyphenol R-1 can serve as a promising pharmacological modulator of calcium transport and synaptic plasticity in hypothyroid and related neurodegenerative conditions.

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