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Corrective Effect Of Polyphenol R-4 On Calcium Homeostasis And Synaptosomal Function In Rats With Experimentally Induced Hypothyroidism

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Abstract: Background: Hypothyroidism is an endocrine disorder characterized by reduced thyroid hormone production, leading to impaired neuronal excitability and calcium signaling.

Objective: This study investigates the corrective effect of polyphenol R-1 on calcium-dependent mechanisms of neuronal activity under experimental hypothyroidism.

Methods: Hypothyroidism was induced in male outbred rats (220–250 g) by oral administration of mercazolil (2.5 mg/100 g) for 21 days. Behavioral, biochemical, and synaptosomal calcium changes were evaluated. Calcium concentrations were measured using Fluo-4 AM fluorescence on a USB 2000 spectrofluorimeter. Polyphenol R-4 (50 mg/kg, intraperitoneal) was administered for 10 days after hypothyroidism induction.

Results: Hypothyroid rats showed lethargy, reduced exploratory activity, decreased T3/T4 levels, elevated TSH, and diminished intracellular [Ca^{2+}]in synaptosomes. Treatment with R-4 significantly normalized these changes, restoring calcium levels, metabolic parameters, and cognitive behavior.

Conclusion: Polyphenol R-4 demonstrates a neuroprotective effect by stabilizing calcium homeostasis through modulation of NMDA receptor and VGCC activity, suggesting its potential as a pharmacological regulator of calcium signaling in hypothyroid-associated neurodegeneration.

Keywords: Hypothyroidism; polyphenol R-4; synaptosomes; calcium homeostasis; NMDA receptors; neuroprotection.

Introduction: Hypothyroidism leads to systemic and neurological impairments due to insufficient thyroid hormone (T3 and T4) synthesis [1]. Thyroid hormones regulate neuronal differentiation, energy metabolism, and synaptic plasticity [2–4]. Their deficiency causes neurotransmitter imbalance, oxidative stress, and impaired calcium signaling, resulting in cognitive and

behavioral deficits [5].

Calcium ions (Ca^{2+}) are central to neurotransmission and synaptic vesicle release. Their intracellular regulation depends on NMDA receptors, voltage-gated calcium channels (VGCCs), and Ca2+-ATPases [6]. In hypothyroidism, downregulation of these components leads to decreased $[Ca^{2+}]_i$n and disrupted neuronal signaling [7].

Polyphenolic compounds have recently emerged as neuroprotective agents due to their ability to modulate receptor activity and calcium transport while reducing oxidative stress [8]. Among them, polyphenol R-4 has demonstrated unique calcium-regulatory properties in neuronal tissues [9-11].

This study investigates the corrective role of R-4 in restoring calcium homeostasis and cognitive functions in rats with experimentally induced hypothyroidism.

METHODS

Experimental Animals

Adult male outbred rats (220–250 g) were housed under standard laboratory conditions (22 ± 2°C, 12 h light/dark cycle) with free access to food and water. All procedures complied with the European Convention for the Protection of Vertebrate Animals (Directive 2010/63/EU).

Induction of Hypothyroidism

Rats received mercazolil (2.5 mg/100 g body weight) orally via gastric tube once daily for 21 days [12]. Control rats received distilled water. Behavioral and biochemical assays confirmed hypothyroidism development.

Behavioral Analysis

Behavioral changes were assessed using the Open Field Test (McGraw scale). Parameters recorded included:

- Horizontal movement (sector crossings)
- Vertical movement (rearing)
- Grooming frequency
- Burrow reflex activity

Reduced movement and grooming indicated central nervous system suppression.

Hormone and Biochemical Analysis

Serum T3 and T4, and TSH levels were measured using HumaReader HS (Human GmbH, Germany). Biochemical assays of brain homogenates included glucose, triglycerides, cholesterol, total protein, AST, and ALT activity.

Synaptosome Isolation

Synaptosomes were isolated from rat brains using the method of Cotman et al. [13]. Brains were homogenized in 0.32 M sucrose–Tris–EDTA buffer (pH 7.4) and centrifuged (4500 rpm, 10 min; 14,000 rpm, 20 min). The final pellet contained purified synaptosomes.

Measurement of Intracellular Calcium

Intracellular calcium concentration was determined using Fluo-4 AM (5 μM) fluorescence [14]. Synaptosomes were incubated with Fluo-4 AM for 30 min at 37°C, washed, and analyzed using a USB 2000 spectrofluorimeter (Ocean Optics, USA) at λ_ex = 488 nm and λ_em = 506 nm.

Polyphenol R-4 Administration

After hypothyroidism induction, a test group received R-4 (50 mg/kg) daily for 10 days. Control and untreated model groups received saline injections.

Statistical Analysis

All data are expressed as mean ± SEM (M ± m). Statistical significance was assessed using Student's t-test ($p < 0.05$ considered significant).

RESULTS

Behavioral Findings

Mercazolil-treated rats displayed decreased locomotor and exploratory activity, indicating CNS suppression. R-4 administration improved movement and grooming, approaching control values (Table 1).

Table 1. Behavioral activity parameters in the Open Field Test.

Parameter	Control	Hypothyroid	Hypothyroid + R-4
Horizontal movement	100 ± 5	54 ± 6	88 ± 5
Grooming frequency	8 ± 1	3 ± 0.5	7 ± 0.8

Hormonal Analysis

Hypothyroidism decreased T3 and T4 levels, while TSH increased. Polyphenol R-4 normalized hormone levels (Table 2).

Table 2. Thyroid hormone levels in control and treated groups.

Group	T_3 (ng/ml)	T_4 (ng/ml)	TSH (μIU/mL)

Control	1.50	91.5	2.1
Hypothyroid	0.675	82.5	4.5
Hypothyroid + R-4	1.42	89.0	2.5

Calcium Levels in Brain Synaptosomes

A significant reduction (~40%) in intracellular $[Ca^{2+}]_{in}$ was observed in hypothyroid rats compared to controls ($p < 0.01$). Treatment with R-4 restored calcium levels near normal (Fig. 1).

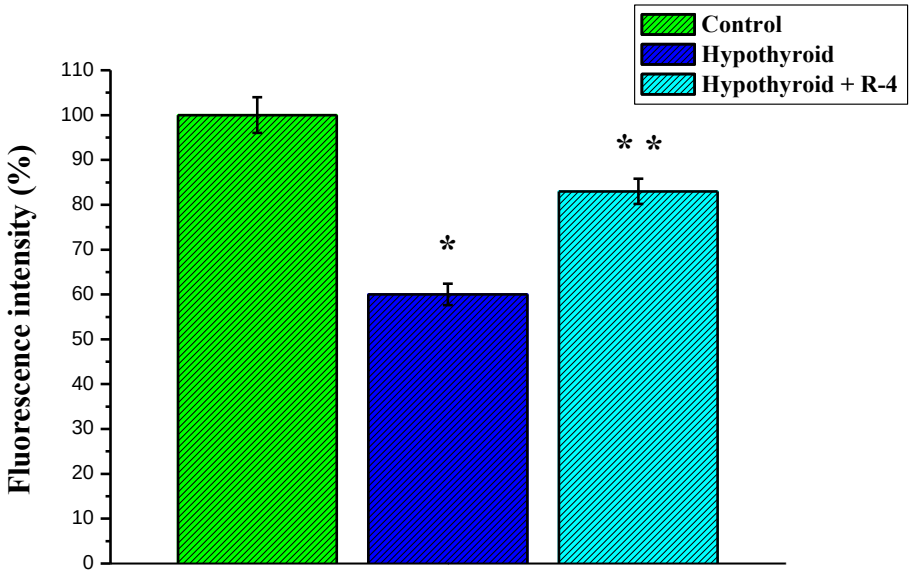

Figure 1. Intracellular calcium concentration ($[Ca^{2+}]_{in}$) in rat brain synaptosomes: 1 – Control; 2 – Hypothyroid; 3 – Hypothyroid + R-4. (*p<0.05; *p<0.01; n=6).

Biochemical Changes

Hypothyroidism increased AST and ALT activity, indicating hepatic and metabolic stress. R-4 reduced these enzyme levels and normalized glucose and protein concentrations, suggesting systemic metabolic correction.

DISCUSSION

Thyroid hormone deficiency impairs calcium regulation by suppressing VGCC and NMDA receptor expression, leading to decreased calcium influx and neurotransmitter release. These findings agree with reports showing reduced excitatory neurotransmission and mitochondrial dysfunction in hypothyroidism [15,16].

Polyphenol R-4 restored calcium homeostasis, likely through:

1. Allosteric modulation of NMDA receptors and VGCCs, enhancing calcium permeability.

2. Antioxidant and mitochondrial protective activity, maintaining ATP-dependent calcium transport.

3. Upregulation of Ca^{2+}-ATPase activity, normalizing intracellular calcium gradients.

Thus, R-4 effectively counteracts hypothyroidism-induced synaptosomal dysfunction, demonstrating therapeutic potential for neuroendocrine disorders.

Scientific Novelty

1. Demonstration of calcium deficiency in synaptosomes as a key factor of neuronal dysfunction in hypothyroidism.

2. Identification of polyphenol R-4 as a calcium-modulating neuroprotective agent.

3. Establishment of a validated rat hypothyroidism model for testing calcium-regulating compounds.

CONCLUSION

The experimental model of hypothyroidism induced by mercazolil produces pronounced neurochemical and behavioral alterations, including calcium depletion in brain synaptosomes. Polyphenol R-4 corrects these disturbances, restoring hormonal, biochemical, and neuronal parameters. The compound's ability to modulate calcium transport and receptor activity highlights its promise as a pharmacological modulator

for hypothyroid-related neurodegeneration.

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