Mechanisms of L-Triiodothyronine-Induced Inhibition of Synaptosomal Na\(^+\)-K\(^+\)-ATPase Activity in Young Adult Rat Brain Cerebral Cortex

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Abstract

The role of thyroid hormones (TH) in the normal functioning of adult mammalian brain is unclear. Our studies have identified synaptosomal Na\(^+\)-K\(^+\)-ATPase as a TH-responsive physiological parameter in adult rat cerebral cortex. L-triiodothyronine (T\(_3\)) and L-thyroxine (T\(_4\)) both inhibited Na\(^+\)-K\(^+\)-ATPase activity (but not Mg\(^2+\)-ATPase activity) in similar dose-dependent fashions, while other metabolites of TH were less effective. Although both T\(_3\) and the β-adrenergic agonist isoproterenol inhibited Na\(^+\)-K\(^+\)-ATPase activity in cerebrocortical synaptosomes in similar ways, the β-adrenergic receptor blocker propranolol did not counteract the effect of T\(_3\). Instead, propranolol further inhibited Na\(^+\)-K\(^+\)-ATPase activity in a dose-dependent manner, suggesting that the effect of T\(_3\) on synaptosomal Na\(^+\)-K\(^+\)-ATPase activity was independent of β-adrenergic receptor activation. The effect of T\(_3\) on synaptosomal Na\(^+\)-K\(^+\)-ATPase activity was inhibited by the α\(_2\)-adrenergic agonist clonidine and by glutamate. Notably, both clonidine and glutamate activate G\(_\alpha\)-proteins of the membrane second messenger system, suggesting a potential mechanism for the inhibition of the effects of TH. In this paper, we provide support for a nongenomic mechanism of action of TH in a neuronal membrane-related energy-linked process for signal transduction in the adult condition.

1. Introduction

Thyroid hormones (TH) exert major influences on the growth and development of the mammalian brain through specific nuclear receptor-mediated gene expression. Although several different isoforms of nuclear receptors for TH have been described in adult mammalian brain, their physiological function is quite unclear [1–4]. Still, adult onset of dysthyroidism develops a number of functional, neurological and psychological manifestations in humans [5–7]. In contrast to the developing brain, most of the changes resulting from hormone variations in the adult condition are reversible with the proper adjustment of circulatory TH [5–7].

Recent evidence has demonstrated that L-triiodothyronine (T\(_3\)) is distributed, concentrated, and metabolized in the synaptosomal fraction of adult rat cerebral cortex [5, 8, 9]. Specific T\(_3\)-binding sites have also been described in cerebrocortical synaptosomes [10, 11] and a graded binding of T\(_3\) to its synaptosomal receptor binding sites has been correlated with the corresponding inhibition of the Na\(^+\)-K\(^+\)-ATPase activities in adult rat brain [11]. TH rapidly alters \textit{in vitro} phosphorylation of synaptosomal proteins in a dose-dependent fashion [12]. TH levels are also altered in adult rat brain in different thyroid conditions [9]. TH enhances calcium entry in adult rat brain synaptosomes [13–15], in hypothryroid mouse brain [16], and in single rat myocytes [17].

However, there is a lack of clear understanding of the mechanism(s) of action of TH in the regulation of synaptic functions in adult neurons. The present study investigates the pathways of T\(_3\)-mediated signaling from its binding to the synaptosomal membrane receptors to the subsequent
of second messenger system components that ultimately affect the further downstream effector molecule, the Na\(^+\)-K\(^+\)-ATPase. In this paper, we hypothesize a nongenomic mechanism of action of TH in neuronal membrane-related energy-linked process(es) for signal transduction in adult condition. We have used \(\alpha\)- and \(\beta\)-adrenoceptor agonists and antagonists for modulation of the activity of \(G\text{\textsubscript{RI}}\) and \(G\text{\textsubscript{R}}\)-proteins of the membrane adenylate cyclase system. Portions of this work have appeared elsewhere in a preliminary form [18, 19].

2. Materials and Methods

2.1. Materials. The following compounds were purchased from Sigma Chemical Company, USA: bovine serum albumin (BSA), clonidine hydrochloride (CLO), disodium-ATP, isoproterenol hydrochloride (ISO), 2-mercaptoethanol, ouabain (BSA), clonidine hydrochloride (CLO), disodium-ATP, from Sigma Chemical Company, USA: bovine serum albumin (BSA), clonidine hydrochloride (CLO), disodium-ATP, sodium glutamate, 3,5,3'-isoproterenol hydrochloride (ISO), 2-mercaptoethanol, ouabain (BSA), clonidine hydrochloride (CLO), disodium-ATP, sodium orthovanadate (10nM–2mM) followed by addition of the synaptosomal lysates, each containing 20–50\(\mu\)g synaptosomal protein, at 0°C for 60 minutes in dark. To get a steady-state ouabain binding, both the assay media (i) and (ii), with and without ouabain, respectively, as described above, were preincubated for 60 min at 0°C in the dark, followed by a 5-min incubation at 37°C to equilibrate the temperature. The reaction was started by adding 4 mM Tris-ATP and incubated at 37°C for 10 min. An aliquot of 100\(\mu\)L of 10% sodium dodecylsulfate was added to stop the enzymatic reaction. The inorganic phosphate (Pi) formed was determined in the reaction mixture [21]. Na\(^+\)-K\(^+\)-ATPase activity was calculated as difference in the Pi content between media (i) and (ii) and expressed as \(\mu\)mols P\(_i\)/h/mg protein [22]. The ouabain-sensitive portion of the total ATPase (Na\(^+\)-K\(^+\)-Mg\(^2+\)-ATPase) was determined from the Pi released in the medium (i) minus that in medium (ii). The Pi released from the reaction medium (ii) was used for determination of the synaptosomal Mg\(^2+\)-ATPase activity. Synaptosomal Mg\(^2+\)-ATPase activity, therefore, was assayed as the ouabain-insensitive ATP hydrolysis.

2.2. Treatment of Animals. Adult male Charles Foster rats (3 months old) were housed at 25 ± 1°C in 12 h dark-12 h light conditions and fed ad libitum with standard rat diet and water. The animals were sacrificed by quick decapitation and the brains were removed into ice-cold 250 mM sucrose solution. The cerebral cortices were dissected out for synaptosomal fraction preparation.

2.3. Preparation of Synaptosomes. The synaptosomes from the cerebral cortex were prepared as described previously [20]. Briefly, the cerebral cortex was homogenized (10% weight/volume) in 0.32M sucrose and centrifuged at 1000g for 10 minutes to remove cell debris and nuclei. The supernatant was collected and recentrifuged at 1000g for 10 minutes. The resulting pellet was discarded and the supernatant was layered over 1.2 M sucrose and centrifuged at 34,000g for 50 min at 4°C. The fraction collected between the 0.32 M and 1.2 M sucrose layer was diluted at 1:1.5 with ice-cold bidistilled water, further layered on 0.8 M sucrose, and again centrifuged at 34,000 g for 30 min. The pellet thus obtained was washed and repelleted at 20,000 g for 20 min. Synaptosomal pellets were lysed by suspending in ice-cold bidistilled water to release the occluded Na\(^+\)-K\(^+\)-ATPase activity.

2.4. Assay of Synaptosomal Na\(^+\)-K\(^+\)-ATPase Activity. Synaptosomal Na\(^+\)-K\(^+\)-ATPase activity was assayed as ouabain-sensitive ATP hydrolysis in reaction mixtures of (i) 30 mM imidazole-HCl, 130 mM NaCl, 20 mM KCl, and 4 mM MgCl\(_2\), and (ii) 30 mM imidazole-HCl, 4 mM MgCl\(_2\), and 1 mM ouabain, at pH 7.4. Both the reaction media (i) and (ii) were first preincubated in vitro with or without simultaneous addition of various concentrations of thyroid hormones (T\(_3\), T\(_4\)) and TH-analogue (T\(_2\)) (0.001 nM to 1 \(\mu\)M), adrenergic drugs (1 nM for ISO, PRA, PHE and YOH; 1 nM–100 nM for CLO and PROP), glutamate (100 \(\mu\)M), DB cAMP (1 \(\mu\)M– 5 mM), and sodium orthovanadate (10 nM–2 mM) followed by addition of the synaptosomal lysates, each containing 20–50\(\mu\)g synaptosomal protein, at 0°C for 60 minutes in dark. To get a steady-state ouabain binding, both the assay media (i) and (ii), with and without ouabain, respectively, as described above, were preincubated for 60 min at 0°C in the dark, followed by a 5-min incubation at 37°C to equilibrate the temperature. The reaction was started by adding 4 mM Tris-ATP and incubated at 37°C for 10 min. An aliquot of 1000 \(\mu\)L of 10% sodium dodecylsulfate was added to stop the enzymatic reaction. The inorganic phosphate (Pi) formed was determined in the reaction mixture [21]. Na\(^+\)-K\(^+\)-ATPase activity was calculated as difference in the Pi content between media (i) and (ii) and expressed as \(\mu\)mols P\(_i\)/h/mg protein [22]. The ouabain-sensitive portion of the total ATPase (Na\(^+\)-K\(^+\)-Mg\(^2+\)-ATPase) was determined from the Pi released in the medium (i) minus that in medium (ii). The Pi released from the reaction medium (ii) was used for determination of the synaptosomal Mg\(^2+\)-ATPase activity. Synaptosomal Mg\(^2+\)-ATPase activity, therefore, was assayed as the ouabain-insensitive ATP hydrolysis.

2.5. Measurement of Protein. Synaptosomal protein content was measured using bovine serum albumin as a standard [23].

2.6. Statistical Analysis. Results are expressed as the mean ± SEM of 3–4 separate experiments or as mentioned. Each experiment was made from six rats. The statistical analysis of the data was performed by Student’s t-test, considering \(P < 0.05\) as the significance level. The data for multiple groups were also analyzed by one-way ANOVA followed by Student Newman-Keuls post-hoc comparisons using SigmaStat software. Nonlinear regression analysis was performed using GraphPad Prism software.

3. Results

3.1. Effects of T\(_3\) and Metabolites on Na\(^+\)-K\(^+\)-ATPase Activity. In vitro addition of various doses of T\(_3\) to the synaptosomal fraction (which is devoid of cell nuclei) confirmed our previous observation [11] and showed nearly the same trend of a dose-dependent inhibition (IC\(_{50}\) = 166.4 ± 55.0 pM; maximal inhibition = 63.2 ± 3.4% at 95% confidence levels) of Na\(^+\)-K\(^+\)-ATPase activity. No significant effect of T\(_3\) was noticed on the Mg\(^2+\)-ATPase specific activity (Figure 1). T\(_4\) had a similar inhibitory effect as T\(_3\) on Na\(^+\)-K\(^+\)-ATPase activity (IC\(_{50}\) = 77.2 ± 31.8 pM; maximal inhibition = 66.5 ± 7.2%), while T\(_2\) had minimal effects (Figure 2). Furthermore, the same range of doses (10\(^{-12}\)–10\(^{-8}\) M) of r-T\(_3\) did not inhibit either Na\(^+\)-K\(^+\)-ATPase or Mg\(^2+\)-ATPase activities (data not shown).

3.2. Effect of T\(_3\) and \(\beta\)-Adrenergic Agonists/Antagonists on Na\(^+\)-K\(^+\)-ATPase Activity. Equimolar doses (1 nM) of T\(_3\) and
Figure 1: Inhibitory effect of various doses (0.001 nM–100 nM) of T₃ on synaptosomal Na⁺-K⁺-ATPase or Mg²⁺-ATPase activity, in vitro. The data are represented as mean ± SEM of ten separate experiments, taking six animals in each group. The vertical lines denote SEM. Filled circles indicate Na⁺-K⁺-ATPase activity while filled triangles indicate Mg²⁺-ATPase activity.

Figure 2: Inhibitory effect of various doses (0.001 nM–10 nM) of T₃ or T₂ on synaptosomal Na⁺-K⁺-ATPase activity, in vitro. The data are represented as mean ± SEM of four separate experiments, taking six animals in each group. The vertical lines denote SEM. Filled circles indicate effects of T₃ on Na⁺-K⁺-ATPase activity while filled squares indicate effects of T₂.

The nonselective β-adrenergic agonist ISO were added separately in vitro, inhibited the Na⁺-K⁺-ATPase enzyme activity by 41.3% and 42.6%, respectively (Figure 3). The nonselective β-adrenergic antagonist PROP alone did not alter the enzyme activity at different doses (1 nM, 10 nM, and 100 nM). The inhibitory action of ISO (1 nM) on the Na⁺-K⁺-ATPase activity was counteracted by PROP (1 nM), whereas PROP could not block T₃-mediated inhibition of the enzyme activity. Instead PROP potentiated the T₃-mediated inhibition of the enzyme activity in a dose-dependent manner. Significant differences in the potentiation of the T₃ effect (1 nM) by PROP on Na⁺-K⁺-ATPase activity and its modulation by a β-adrenergic receptor agonist (ISO) and a β-adrenergic receptor antagonist (PROP) in vitro. A half-maximally effective dose of T₃ (1 nM) was chosen from the dose-response curve for T₃ in Figure 1. The data are represented as mean ± SEM of five separate experiments taking six animals in each group. *P < 0.001, compared to the control group. †P < 0.001 and ‡P < 0.05, compared to T₃ (1 nM) + PROP (100 nM) group (one-way ANOVA followed by Newman-Keuls test). The vertical lines denote SEM.

3.3. Effects of T₃ and α-Adrenergic Agonists/Antagonists on Na⁺-K⁺-ATPase Activity. The effects of in vitro addition of 1 nM doses of PHE (selective α₁-adrenergic receptor agonist) and PRA (α₁-adrenergic receptor antagonist) on synaptosomal Na⁺-K⁺-ATPase activity or Mg²⁺-ATPase activity were minimal (Figure 4). Furthermore, 1 nM doses of PHE or PRA did not alter the inhibitory effect of 1 nM T₃ on Na⁺-K⁺-ATPase activity, nor did it change the Mg²⁺-ATPase activity, in vitro (Figure 4).

Similarly, in vitro addition of CLO (α₂-adrenergic agonist) at different doses did not elicit significant changes in the synaptosomal Na⁺-K⁺-ATPase activity (Figure 5). However, when CLO was added in the presence of an equimolar dose of T₃, the inhibitory effect of T₃ on the Na⁺-K⁺-ATPase activity was completely counteracted. The effect of T₃ on the enzyme activity remained prominent at a 100 nM dose of T₃ (100 nM T₃: 10.29 ± 0.2 μmols Pᵢ/h/mg protein;
ATPase activity by $\alpha$-doses for PHE and PRA used for the experiments, taking six animals in each group. The vertical lines denote SEM.

Control: $26.22 \pm 0.2 \mu$moles P$_i$/h/mg protein) along with 1 nM CLO (100 nM $T_3$ + 1 nM CLO: 15.23 $\pm$ 0.4 $\mu$moles P$_i$/h/mg protein); however, 1 nM CLO attenuated the effect of $T_3$ (100 nM) by 32% more towards the control value (data not shown graphically). The $\alpha$-adrenergic receptor antagonist YOH also inhibited synaptosomal Na$^+$-K$^+$-ATPase activity (Figure 5). inhibition of the enzyme activity in the presence of both 1 nM $T_3$ and 1 nM YOH was found to be intermediate between the levels of inhibition by either compound alone, although there were no significant differences between these groups (Figure 5).

3.4. Effect of $T_3$ and Glutamate on Na$^+$-K$^+$-ATPase Activity. In vitro addition of 100 tM glutamate alone did not alter the synaptosomal Na$^+$-K$^+$-ATPase activity compared to control values, whereas, addition of 100 tM glutamate showed complete attenuation of $T_3$ (10 nM)-mediated inhibition of synaptosomal Na$^+$-K$^+$-ATPase activity in adult rat cerebral cortex (Figure 6). A higher dose of $T_3$ (10 nM) was chosen, in order to test the effect of glutamate against a greater inhibitory action on the Na$^+$-K$^+$-ATPase activity.

3.5. Effect of DB cAMP and $T_3$ on Na$^+$-K$^+$-ATPase Activity. To study the effect of DB cAMP on modulation of Na$^+$-K$^+$-ATPase activity by $T_3$, first a dose response experiment with various concentrations of DB cAMP (0.001 mM to 5 mM) was performed. In vitro addition of DB cAMP showed a typical sigmoidal curve with gradual decrease in the Na$^+$-K$^+$-ATPase activity to a maximal inhibition at 0.2 mM (Figure 7(a)). From this standardization, we chose to use a 0.2 mM final concentration of DB cAMP for further experiments. In vitro addition of DB cAMP (0.2 mM) with and without various doses of $T_3$ (0.001 nM–10 nM) was examined for effects on Na$^+$-K$^+$-ATPase activity (Figure 7(b)). $T_3$-induced inhibition of synaptosomal Na$^+$-K$^+$-ATPase activity was further depressed in the presence of 0.2 mM DB cAMP. However, the two curves appeared to converge at the highest doses of $T_3$.

3.6. Influence of Sodium Orthovanadate on Modulation of Na$^+$-K$^+$-ATPase Activity by $T_3$. The in vitro effect of sodium orthovanadate, a protein tyrosine phosphatase inhibitor, was examined in cerebrocortical synaptosomes. The cerebrocortical synaptosomes were treated with a fixed dose of $T_3$ (10 nM) with or without different doses of sodium o-vanadate (Figure 8). A higher dose of $T_3$ (10 nM) was chosen from the $T_3$ dose-response curve, considering its greater inhibitory action on the Na$^+$-K$^+$-ATPase activity. $T_3$ caused an inhibition of Na$^+$-K$^+$-ATPase specific activity, and this effect was enhanced by sodium orthovanadate in a dose-dependent way. In general, the effects of sodium orthovanadate and $T_3$ appeared to be additive until the Na$^+$-K$^+$-ATPase specific activity was completely inhibited.

4. Discussion

The objective of the present investigation was to search for possible mechanisms for the inhibition by TH of synaptosomal Na$^+$-K$^+$-ATPase activity in adult rat cerebral cortex.

Initial studies examined the specificity of the effect according to the pattern of iodination of the hormone derivatives (Figures 1 and 2). In vitro inhibitory effect of $T_3$ on synaptosomal Na$^+$-K$^+$-ATPase activity supported our previous observation and showed nearly the same trend of a dose-dependent inhibition of Na$^+$-K$^+$-ATPase activity [11]. In addition to our earlier report, the current study showed an insignificant effect of $T_3$ on the synaptosomal Mg$^{2+}$-ATPase specific activity (Figure 1). In vitro addition of $T_3$ also indicated similar pattern of inhibitory influence on the synaptosomal Na$^+$-K$^+$-ATPase activity, like the effect of $T_3$, with no significant changes on the Mg$^{2+}$-ATPase activity. The effects of TH on Na$^+$-K$^+$-ATPase activity seemed to be
specific for compounds with 2 iodine atoms on the inner ring, as T₁ and rT₃ were without activity in the current studies. T₃ was less potent than T₄. It is consistent with reports of the relative affinities of the two compounds for a cell surface receptor, integrin αᵥβ₃ known to mediate a variety of nongenomic effects of THs [24].

Binding of T₄ to integrin αᵥβ₃ causes internalization of the receptor and nongenomically promotes phosphorylation of mitogen-activated protein kinase/extracellular regulated kinase 1 and 2 (MAPK/ERK₁/2) in the CV-1 line of monkey fibroblasts [24]. A similar mechanism seems likely in chick chorioallantoic membrane [25]. Following the internalization of the integrin αᵥβ₃, the αᵥ monomer is translocated to the nucleus, where it may transcriptionally regulate expression of protein [26]. TH causes lungs to rapidly (within hours) increase alveolar fluid clearance [27] and to express increased Na⁺-K⁺-ATPase protein by a MAPK/ERK₁/2-dependent pathway [28]. Note, however, that the current finding of an immediate effect to decrease Na⁺-K⁺-ATPase activity could not be due to a mechanism involving transcriptional regulation, since the synaptosomal preparation is devoid of cell nuclei. It is also suggested that some of the effects of T₃ stimulation of the integrin αᵥβ₃ could be more direct than the nuclear interaction [29].
a $\beta$-adrenergic receptor-mediated event, potentially coupled to $G_\text{i}$-protein. However, PROP was completely unable to block $T_3$-mediated inhibition of synaptosomal Na$^+$-K$^+$-ATPase activity. This clearly indicated that $T_3$-mediated inhibition of the enzyme activity was not coupled to $\beta$-adrenergic receptor, but rather, may have had a similar effect through another kind of receptor. The augmentation of the $T_3$ effect by PROP appeared to be a type of synergistic action, the mechanism of which remains unclear at present. Increased activity of adenylate cyclase caused by THs, independent of propranolol blockade, has been shown in cultured cerebral cells from embryonic mice, suggesting that the effect of $T_3$ was not mediated through a $\beta$-adrenergic-dependent system [34]. The $T_3$-induced increase in sodium current in neonatal rat myocytes also could not be blocked by PROP, whereas it was antagonized by amiodarone, a nonspecific blocker of $\beta$-adrenoceptor, suggesting that the effects were not mediated through $\beta$-adrenergic signaling pathways [35]. However, $\beta$-adrenoceptor blockade by chronic subcutaneous delivery of PROP for 14 days has been shown to downregulate levels of TH receptor TR $\alpha_1$-mRNA and $\beta_2$-mRNA in mouse heart, which may influence the genomic effect of the hormone [36].

Next, we wanted to check for the role of an $\alpha_1$-adrenergic receptor agonist and antagonist. Agonists for the $\alpha_1$-adrenergic receptor mediate their actions through $G_\text{q}$ protein followed by activation of phospholipase C and subsequent production of the second messengers inositol triphosphate and diacylglycerol, an activator of protein kinase C [37]. Neither PHE (selective $\alpha_1$ agonist) nor PRA ($\alpha_1$ antagonist) has had an influence on Na$^+$-K$^+$-ATPase activity. Furthermore, neither compound interacted with the effects of $T_3$. Mg$^{2+}$-ATPase activity remained unaltered when treated with either of these $\alpha_1$-adrenergic drugs (agonist and antagonist) and $T_3$, alone or in combination (Figure 4). These results suggest that the effects of $T_3$ on Na$^+$-K$^+$-ATPase activity do not share common mechanisms with $\alpha_1$-receptors.

On the other hand, CLO, an $\alpha_2$-adrenergic receptor agonist (Figure 5), and glutamate (Figure 6), possibly acting via a metabotropic glutamate receptor (mGluR), blocked $T_3$-induced inhibition of Na$^+$-K$^+$-ATPase activity. Neither CLO nor glutamate showed any significant effect on the Na$^+$-K$^+$-ATPase activity in rat hippocampus and frontal cortex homogenates [38]. One possibility would be that the counteraction of the effect of $T_3$ on synaptosomal Na$^+$-K$^+$-ATPase by CLO and glutamate might be mediated through the inhibition of adenylate cyclase activity with the activation of inhibitory G-protein ($G_\text{i}$) followed by the inhibition of cAMP synthesis and the protein phosphorylation cascade mechanism. It is well known that $\alpha_2$-adrenergic agonists act through stimulation of $G_\text{i}$-protein [18, 19, 39, 40].

Association of the glutamate transporter with Na$^+$-K$^+$-ATPase in synaptosomes has been implicated by their correlated regulation via protein kinases [41]. Glutamate also has been reported to inhibit adenylate cyclase activity in rat hippocampal synaptosomes [39, 40, 42, 43], as well as in striatal and cerebrocortical neurons, both in intact cells and membranes [40] via metabotropic glutamate receptors (mGluRs), which are coupled to effector systems through GTP binding proteins. In fact, in the nucleus tractus solitarius of adult brain, it was shown that an antibody to the $G_i$ inhibited the effects of mGluRs [44]. mGluR$_1$ and mGluR$_5$ subtypes are coupled to phosphatidyl inositol hydrolysis/Ca$^{2+}$-signal transduction. mGluR$_1$ has also been shown to stimulate release of arachidonic acid and to increase cAMP formation. The mGluR$_2$, mGluR$_3$, mGluR$_4$, and mGluR$_5$ subtypes appear to be coupled to inhibition

**Figure 7:** Influence of DB cAMP and $T_3$ on synaptosomal Na$^+$-K$^+$-ATPase activity, *in vitro*. (a) Inhibitory effect of various doses of DB cAMP on synaptosomal Na$^+$-K$^+$-ATPase activity, *in vitro*. The data are represented as mean ± SEM of four separate experiments, taking six animals in each group. The vertical bars denote SEM. (b) Interaction of the effects of DB cAMP and $T_3$ on synaptosomal Na$^+$-K$^+$-ATPase activity, *in vitro*. Filled squares indicate effects of graded doses of $T_3$ (0.001 nM–10 nM) alone on Na$^+$-K$^+$-ATPase activity while filled triangles indicate effects of the 0.2 mM dose of DB cAMP with graded doses of $T_3$ (0.1 pM–1 μM).
and protein kinase C has been shown to inhibit this enzymatic activity in shark rectal gland, rat renal cortex, and basolateral membrane vesicles from rat renal cortex [46].

The effect of the protein tyrosine phosphatase inhibitor sodium orthovanadate [47] appeared to be additive to the effect of T₃, implying that there could be a separate mechanism of action of the two compounds (Figure 8). Since vanadate is a blocker of tyrosine phosphatase activity, it also could be speculated that T₃-induced inhibition of Na⁺-K⁺-ATPase activity is further suppressed by synergistic action by vanadate via keeping the enzyme in its phosphorylated form, causing inhibition of its activity. A point to note here is that the α-subunit is the catalytic subunit and its phosphorylation causes inhibition of this enzyme [46]. T₃ appears not to have the inhibitory effect on Na⁺-K⁺-ATPase activity by an influence on phosphatase activity.

5. Conclusion

Our results regarding T₃ action in relation to the inhibition of synaptosomal Na⁺-K⁺-ATPase are consistent with a T₃-synaptosomal membrane component binding site interaction sensitive to the activation of G₅-protein. Such a membrane binding component might interact with a G₅-protein, resulting in increased synthesis of cAMP. The membrane Na⁺-K⁺-ATPase is involved in several aspects of physiological processes. In the neuron, its inhibition is linked with neurotransmitter release [46]. Hence, the present study provides further evidence of a nongenomic membrane-related action of T₃ in the mature mammalian synaptosome. Understanding of the mechanism of action of TH in adult mammalian brain has major implications in the higher mental functions and in the regulation of several neuropsychiatric disorders developed in thyroid dysfunctions in adult humans.

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