Biochemistry and Neurobiology. In the articles "Binding of thymopoietin to the acetylcholine receptor" by K. Venkatasubramanian, T. Audhya, and G. Goldstein; "Calcium dependent effect of the thymic polypeptide thymopoietin on the desensitization of the nicotinic acetylcholine receptor" by F. Revah, C. Mulle, C. Pinset, T. Audhya, G. Goldstein, and J. P. Changeux; and "Evidence for thymopoietin and thymopoietin/\(\alpha\)-bungarotoxin/nicotinic receptors within the brain" by M. Quik, U. Babu, T. Audhya, and G. Goldstein, which appeared in number 10, May 1986; number 10, May 1987; and number 6, March 1991 (83, 3171–3174; 84, 3477–3481; and 88, 2603–2607), respectively, the authors request that the following be noted. Studies by Quik et al. (1) and confirmatory observations by G. Goldstein (Immunobiology Research Institute, Annandale, NJ) and by R. Lukas (Barrow Neurological Institute, Phoenix, AZ; personal communication) show that some research samples of natural or synthetic thymopoietin containing \(\alpha\)-cobratoxin or phospholipase \(A_2\) activity were provided by the Immunobiology Research Institute prior to 1991 and used in research published up to the end of 1992. In view of this contamination, it cannot be concluded from the experiments reported in these papers that thymopoietin interacts acutely with any type of nicotinic receptor, and we must retract these articles. We deeply regret this situation.


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Genetics. In the article "In vivo and in vitro evidence for slipped mispairing in mammalian mitochondria" by Cort S. Madsen, Steven C. Ghivizzani, and William W. Hauswirth, which appeared in number 16, August 15, 1993, of Proc. Natl. Acad. Sci. USA (90, 7671–7675), the authors request that the following correction be noted. On p. 7672, the sentence beginning on line 2 of Results should read as follows: "The porcine repeat domain and flanking regions have been previously sequenced, and the range of repeat copy number variability was determined (refs. 11 and 14; Fig. 1)."
Calcium-dependent effect of the thymic polypeptide thyrompoietin on the desensitization of the nicotinic acetylcholine receptor

(allosteric transitions/myasthenia gravis/thymic hormone)

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ABSTRACT The effects of the thymic polypeptide thyrompoietin (Tpo) on the properties of the nicotinic acetylcholine receptor (AcChoR) were investigated by patch clamp techniques on mouse C2 myotubes and by biochemical assays on AcChoR-rich membrane fragments purified from the Torpedo marmorata electric organ. At high concentrations (>100 nM), Tpo inhibits the binding of cholinergic agonists to the AcChoR in a Ca²⁺-insensitive manner. At lower concentrations (2 nM), Tpo applied on C2 myotubes simultaneously with nondesensitizing concentrations of acetylcholine results in the appearance of long closed times separating groups of openings. This effect depends on the presence of Ca²⁺ in the external medium. Outside-out recordings, performed with various concentrations of EGTA in the intracellular medium, suggest that Ca²⁺ acts on the cytoplasmic face of the membrane after entry through acetylcholine-activated channels. Parallel studies with T. marmorata AcChoR-rich membranes show that in the presence of Ca²⁺ Tpo causes a decrease in the apparent equilibrium dissociation constant of the noncompetitive blocker [3H]phencyclidine, enhances, at low concentrations, the binding of [3H]acetylcholine, and also alters the binding kinetics of the fluorescent agonist 6-(5-dimethylamino-1-naphthalenesulfonamido)-n-hexanoic acid β-(N-trimethylammonium bromide) ethyl ester to the AcChoR. It was concluded that, in the presence of Ca²⁺, Tpo displaces the conformational equilibrium of the AcChoR towards a high-affinity desensitized state and increases the transition rate towards the same state.

The nicotinic acetylcholine receptor (AcChoR) from the vertebrate neuromuscular junction and the fish electric organ is a ligand-regulated ion channel, which undergoes several distinct categories of allosteric transitions (for review, see ref. 1). In addition to the fast opening and closing of the ion channel, the neurotransmitter acetylcholine (AcCho) and a variety of allosteric effectors elicit slower reversible transitions towards desensitized states, in which the AcChoR is refractory to activation. The physiological significance of these desensitized states is still debated. A plausible hypothesis is that they are involved in the regulation of synapse efficacy at the postsynaptic level (2). In a search for endogenous substances distinct from AcCho that may regulate AcChoR desensitization, it was of interest to study the effects of the thymic hormone thyrompoietin (Tpo). Tpo, a polypeptide hormone physiologically secreted by epithelial cells of the thymus, induces differentiation of prothymocytes and affects the functions of mature T cells (3). Studies of the human disease myasthenia gravis, in which thymic abnormalities are associated with muscle weakness and fatigability caused by impaired neuromuscular transmission (4), led to the hypothesis that an autoimmune thymitis present in myasthenia gravis results in hypersecretion of a thymic substance responsible for postsynaptic impairments in cholinergic transmission (5, 6). The detection, in thymic extracts, of a factor causing electromyographically detectable changes in neuromuscular functioning with fatigability at nerve stimulation frequencies of 50 Hz led to the purification and the determination of the complete amino acid sequences of bovine and human Tpos (7–9). Recently, equilibrium binding studies with Torpedo californica AcChoR-rich membrane fragments have shown that Tpo interacts with the AcCho binding site (10). We have now further studied the interaction of bovine Tpo and human synthetic Tpo (STpo) with the AcChoR of C2 mouse myotubes by using patch clamp techniques and with AcChoR-rich membranes from Torpedo marmorata by using a variety of biochemical approaches; we show that this thymic polypeptide regulates AcChoR desensitization.

MATERIALS AND METHODS AcChoR-rich membrane fragments were purified from T. marmorata electric organ as described (11), in the presence of protease inhibitors and chelating agents to limit proteolysis, and were stored in liquid nitrogen. Tpo was purified from bovine thymus as previously reported (7), and STpo was prepared from the human sequence (8) (Peninsula Laboratories, San Carlos, CA). The peptides were stored at 4°C. [3H]AcCho (86 Ci/mmol; 1 Ci = 37 GBq) was from Amersham and [3H]phencyclidine (50 Ci/mmol) was from New England Nuclear. Phencyclidine was a gift from A. Jaganathan (Université Louis Pasteur, Strasbourg, France), and 6-(5-dimethylamino-1-naphthalenesulfonamido)-n-hexanoic acid β-(N-trimethylammonium bromide) ethyl ester (Dns-C6-Cho) was from G. Waksman and B. Roques (Faculté de Pharmacie Université René Descartes, Paris, France). Live T. marmorata were provided by the Biological Station of Arcachon (France).

Single AcCho-activated currents were recorded at room temperature (20–23°C) from cell-attached and outside-out membrane patches (12) obtained from myotubes of the mouse cell-line C2 that were incubated in 140 mM NaCl/4 mM KCl/1 mM CaCl₂/1 mM MgCl₂/10 mM Hepes, pH 7.2. In some experiments, CaCl₂ was omitted and 0.5 mM EGTA was added to the solution. In the cell-attached mode, 200 nM AcCho, either alone or with various concentrations of Tpo (2–200 nM), was added to the pipette-filling solution. For outside-out recordings, the pipette filling solution was 145 mM KCl/4 mM NaCl/1 mM MgCl₂/10 mM Hepes, pH 7.2.

Abbreviations: AcCho, acetylcholine; AcChoR, nicotinic acetylcholine receptor; Tpo, bovine thyrompoietin; STpo, synthetic human thyrompoietin; Dns-C6-Cho, 6-(5-dimethylamino-1-naphthalenesulfonamido)-n-hexanoic acid β-(N-trimethylammonium bromide) ethyl ester.

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containing either 10 mM EGTA and 0.5 mM CaCl₂ or 0.1–0.2 mM EGTA and no Ca²⁺. The external face of the membrane was then successively bathed with 500 nM AcCho and various concentrations of Tpo (2–200 nM). Single channel data were recorded and analyzed as previously described (13).

The equilibrium binding of [³H]AcCho and [³H]phencyclidine in the presence of Tpo or STpo was measured with T. marmorata AcChoR-rich membranes diluted in Torpedo Ringer’s solution with CaCl₂ (250 mM NaCl/5 mM KCl/4 mM CaCl₂/2 mM MgCl₂/5 mM sodium phosphate, pH 7) or without CaCl₂ (no CaCl₂ and 6 mM MgCl₂). For [³H]AcCho binding experiments, the membrane fragments were pretreated for 1 hr with 50 μM diisopropyl fluorophosphate to inhibit acetylcholinesterase. Aliquots (250 μl) were equilibrated with a fixed nonsaturating concentration of [³H]AcCho and various concentrations of Tpo or STpo for at least 1 hr at 4°C and were filtered through presoaked Whatman GF/B filters. The filters were then rapidly washed with 10 ml of the Ringer’s solution, dried, transferred to counting vials, and counted with Biofluor (New England Nuclear) in an LKB 1214 counter. When [³H]phencyclidine binding was measured, bound and free ligand were separated by centrifuging the aliquots (300 μl; 15,000 x g for 10 min). The supernatant was removed and counted; the pellet was resuspended in 100 μl of 10% (vol/vol) Triton X-100 and was counted.

Fluorescence recording and the analysis of Dns-C₆-Cho interactions with AcChoR-rich membranes (millisecond to minute time range) were carried out as described (14).

RESULTS

Effects of Tpo on the Kinetics of AcCho-Activated Single-Channel Currents from Mouse C₂ Cell line. The effects of bovine Tpo on AcCho-activated currents were first tested using the cell-attached mode with myotubes from the mouse C₂ cell line. When the patch membrane was held at 100 mV hyperpolarized from rest, most AcCho-activated currents had amplitudes of ~5 pA (slope conductance = 34 ± 3 pS), and most openings were exponentially distributed with a mean open time of 32 ± 2 ms (see ref. 13) (Fig. 1A). Thus, they correspond to channels of the so-called “embryonic” type (15). When a nondesensitizing concentration of AcCho (200 nM) was included in the patch pipette, the closed time distribution appeared as the sum of two exponentials. The fast component (time constant of a fraction of a millisecond) reflects the transient closures within channel openings (16) and was not further analyzed in this study. The slower component represents separate activations of the ion channel by AcCho and depends on the number of activable channels present in each membrane patch. Its time constant T₁, therefore, varied from patch to patch at a given AcCho concentration (50–200 ms for 200 nM AcCho). Addition of Tpo (2–200 nM) to the AcCho pipette solution affected neither the single channel slope conductance (not shown) nor the open time in the range of Tpo concentrations investigated (32 ± 3 ms for 2–20 nM Tpo; n = 6 patches). Furthermore, when Tpo (100 nM; n = 3 patches) was included in an AcCho-free pipette solution, it never activated AcChoR channel currents.

When low concentrations of Tpo (2 nM) were applied simultaneously with nondesensitizing concentrations of AcCho, the appearance of long closed times separating groups of openings was observed (Fig. 1A2). The distribution of closed times (excluding transient closures) could be fitted by two exponentials (Fig. 1A2). For T₁ values between 80 and 120 ms, the time constant T₂, corresponding to the long gaps between groups of openings, was on the order of seconds (T₂ = 1200 ± 50 ms; n = 3 patches).

At higher concentrations of Tpo (200 nM), nearly all AcChoR channels present in the patch rapidly inactivated (Fig. 1B). The channel opening frequency showed a marked decrease over the first minutes of recordings (91 ± 3% decrease; n = 3 patches; 4 min after seal formation—i.e., after application of both compounds on the membrane) with a half decay time of ~1 min. Under these conditions, measurement of closed and open times was rendered difficult. Tpo thus favors the appearance of long periods during which the AcChoR is refractory to activation.

We then used the cell-attached configuration to test the effect of extracellular Ca²⁺ on Tpo action. In a Ca²⁺-free external medium, addition of Tpo (2–20 nM; n = 4 patches) to the AcCho pipette solution did not produce long closings.
between groups of openings (Fig. 1A1), so that the closed time distribution could be fitted by a single exponential. The addition of higher concentrations of Tpo (200 nM) to the AcCho pipette solution led to a progressive decrease in the opening frequency (39 ± 3%; n = 3 patches; 4 min after seal formation), but this decrease was significantly smaller than that observed when Ca\(^{2+}\) was present in the extracellular medium (Fig. 1B). In control experiments, with or without Ca\(^{2+}\) in the external medium, channel opening frequency remained stable over several minutes.

Thus, Ca\(^{2+}\) influences the effect of Tpo either directly (on the external face of the membrane), or indirectly (on its cytoplasmic face) as a consequence of Ca\(^{2+}\) entry through AcChoR channels (17). Outside-out recordings were used to test the latter hypothesis under conditions of rapid buffering of Ca\(^{2+}\) entry (10 mM EGTA and 0.5 mM Ca\(^{2+}\) added to the internal face medium) or under conditions where internal Ca\(^{2+}\) concentrations were permitted to fluctuate (0.1–0.2 mM EGTA and no Ca\(^{2+}\)) (Fig. 2). With 10 mM EGTA present on the internal face of the membrane, no long closures between groups of openings were observed (n = 7 patches) at Tpo concentrations ranging from 2 to 200 nM. However, the relative frequency of openings, as compared to the control (500 nM AcCho alone), decreased with increasing Tpo concentrations (from ~5% decrease for 2 nM Tpo to a 50% decrease for 200 nM Tpo) (Fig. 2A). There was a shift of closed time distribution towards higher values of \(T_1\) without the appearance of an additional component for long closed times (Fig. 2A). This effect was reversed in a few minutes and is consistent with the previously reported competitive blocking action of Tpo (10).

When low concentrations of EGTA (0.1–0.2 mM) were used to chelate Ca\(^{2+}\) entering through AcChoR channels, Tpo effects on AcChoR currents were similar to those observed in the cell-attached configuration, except for a shift in the concentration at which Tpo was effective (Fig. 2B). Extensive washing of the membrane patch with AcCho alone in the medium led to a slow and at least partial recovery. Ca\(^{2+}\) ions thus enhance the effect of Tpo on the AcChoR when it is present on the cytoplasmic side of the membrane. Biochemical assays were performed to determine to what extent the appearance of long closed times is related to desensitization of the AcChoR (18).

**Equilibrium Binding Experiments with Tpo and AcChoR-Rich Membranes from T. marmorata.** At equilibrium, in the absence of Ca\(^{2+}\) and with a nonsaturating fixed total concentration of \(^{[3]H}\)AcCho, Tpo caused a concentration-dependent decrease of bound \(^{[3]H}\)AcCho, consistent with a competitive interaction of Tpo with the AcCho binding site (10). In the presence of 4 mM Ca\(^{2+}\), however, Tpo exhibited a more complex action (Fig. 3A). In the 10–100 nM range, Tpo enhanced \(^{[3]H}\)AcCho binding up to 80%, whereas at concentrations >100 nM, Tpo displaced bound \(^{[3]H}\)AcCho, as was found in the absence of Ca\(^{2+}\). Enhancement of \(^{[3]H}\)AcCho binding did not occur when Ca\(^{2+}\) was omitted or replaced by Mg\(^{2+}\).

Under conditions where \(^{[3]H}\)phencyclidine binds to its high-affinity site (19), neither Tpo nor STpo displaced bound \(^{[3]H}\)phencyclidine, but both of them caused a Ca\(^{2+}\)-dependent increase in \(^{[3]H}\)phencyclidine binding. Fig. 3B shows that in the presence of 4 mM Ca\(^{2+}\), saturating concentrations of STpo decreased the equilibrium dissociation constant of \(^{[3]H}\)phencyclidine from 4.0 to 2.1 \(\mu M\) without significantly changing the number of binding sites. Thus, under equilibrium conditions, STpo (and Tpo) exerts calcium-dependent positive allosteric effects on the binding of \(^{[3]H}\)AcCho and \(^{[3]H}\)phencyclidine to their respective sites.

**Effects of Tpo on the Rapid Kinetics of the Interaction of the Fluorescent Agonist Dns-C6-Cho with AcChoR-Rich Membranes from T. marmorata.** Rapid mixing of membranes with

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**Fig. 2.** Distribution of closed AcChoR channel life times obtained from outside-out membrane patches (membrane potential = 80 mV) in the presence of 10 mM (A) or 0.2 mM (B) EGTA in the intracellular medium. The same patch was successively exposed to 500 nM AcCho (Left) and 500 nM AcCho plus 20 nM Tpo (Right).

**Fig. 3.** (A) Effect of Tpo on AcCho binding to the AcChoR. AcChoR-rich membranes purified from the *T. marmorata* electric organ (final concentration of \(^{125}\)I-labeled \(\alpha\)-bungarotoxin binding sites \(\approx 100\) nM) were incubated with a fixed nonsaturating concentration of \(^{[3]H}\)AcCho and Tpo in incubation medium containing either 0 or 4 mM Ca\(^{2+}\). The radioactivity corresponding to the \(^{[3]H}\)AcCho specifically retained on the filter after washing is plotted as a function of the total Tpo concentration. (B) Effect of STpo on \(^{[3]H}\)phencyclidine binding to the AcChoR. AcChoR-rich membranes purified from the *T. marmorata* electric organ (concentration of \(^{125}\)I-labeled \(\alpha\)-bungarotoxin binding sites \(\approx 350\) nM) were incubated with various concentrations of \(^{[3]H}\)phencyclidine (isotopically diluted to \(\approx 2 \times 10^{12}\) cpm/mmol), in the absence or presence of STpo, in medium containing 0 or 4 mM Ca\(^{2+}\). The radioactivity specifically retained in the pellet and that remaining in the supernatant after centrifugation are plotted in a double-reciprocal representation.
Intermediate and (iii) slow processes progress in the 100 ms to minute time scale and are interpreted on the basis of the interconversion of the AcChoR from its resting R state into states of, respectively, intermediate (L) and high (D) affinity for agonists. Figs. 4 and 5 show that Tpo and STpo modified these kinetics in several ways. The amplitude of the total fluorescence signal decreased to negligible values with increasing Tpo concentrations. This effect did not depend on the presence of Ca\(^{2+}\) in the medium and most likely resulted from the occupation of the agonist binding site by the peptide.

In addition, both Tpo and STpo markedly affected the kinetics of the fluorescence signal in a Ca\(^{2+}\)-dependent manner (Figs. 4 and 5). In the presence of 220 nM STpo and 4 mM Ca\(^{2+}\), the relative amplitude of the rapid signal as a percentage of the total signal rose from \(\approx 20\%\) to \(\approx 63\%\). Moreover, under the same conditions, the rate of the intermediate transition increased 3.3-fold. None of these effects were observed in the absence of Ca\(^{2+}\) or when Mg\(^{2+}\) was substituted for Ca\(^{2+}\). The effects of STpo remained after treatment of the AcChoR-rich membranes at pH 11 (20). These data are in close agreement with the patch clamp and equilibrium binding data and are consistent with the interpretation that in the presence of Ca\(^{2+}\) Tpo both stabilizes and accelerates the transition to the D state of high-affinity for agonists and noncompetitive blockers (for review, see ref. 1).

This state has been identified as a closed channel and desensitized state of the AcChoR (14, 21).

**DISCUSSION**

Electrophysiological studies with mouse C2 myotubes and biochemical observations with *T. marmorata* AcChoR-rich membranes provide parallel evidence that Tpo and STpo exert at least two distinct types of effects on the AcChoR. First, in agreement with earlier observations (10), Tpo interacts at high concentrations (\(>100\) nM), in a Ca\(^{2+}\)-independent fashion, with the AcCho binding site as a competitive antagonist.

A second type of effect occurs at significantly lower concentrations of Tpo and STpo (2–200 nM) and shows a striking Ca\(^{2+}\) dependence. Single channel recordings with C2 myotubes in the cell-attached mode reveal that at low concentrations Tpo causes the appearance of long closed times with a mean duration of several hundreds of milliseconds and progressively reduces the frequency of channel opening by more than 90% at high concentrations. Such long closed times could be explained by different mechanisms. Whereas a noncompetitive blocking mechanism similar to that of phencyclidine at high concentrations (13, 22) cannot be excluded by these experiments, no change was observed in the mean channel open time (at least at low concentrations of Tpo) as occurs with phencyclidine or chloropromazine. Furthermore, *in vitro* equilibrium binding studies with AcChoR-rich membranes show that Tpo does not compete with \(^{3}H\)phencyclidine bound to its high-affinity site. On the other hand, the occurrence of long-lived closed states separating groups of openings is also observed in the presence of desensitizing concentrations of AcCho (18). Thus, an alternative interpretation is that Tpo favors the desensitization of the AcChoR.

The equilibrium binding experiments and the rapid kinetic studies carried out with the fluorescent agonist Dns-C\(_6\)-Cho and AcChoR-rich membranes support this latter interpretation. At concentrations below those producing detectable occupation of the agonist binding site, Tpo and STpo enhance the equilibrium binding of \(^{3}H\)AcCho and \(^{3}H\)phencyclidine ligands that have a higher affinity for the AcChoR in its desensitized (D) state than in its resting (R) state (11) and increase the relative amplitude of the rapid fluorescence signal. The latter is interpreted as representing the fraction of the AcChoRs in the D state. These two series of data are...
accounted for by a simplified version of the allosteric transition model of the AcChoR (1, 19) where only its R and D conformations are taken into account. For example, in the case of [3H]phencyclidine binding, the apparent dissociation constant is given by $K_{app} = K_D (1 + L_a)/(1 + L_a K_R/K_D)$, where $L_a$ is the isomerization constant between the R and D states in the absence of phencyclidine and $K_R$ and $K_D$ are the respective dissociation constants of phencyclidine for the AcChoR in the D and R states. If one takes the previously determined values of 1.5 $\mu M$ and 7.6 $\mu M$ for $K_D$ and $K_R$, respectively (19), one finds that excess levels (800 nM) of Tpo shift $L_a$ from 3.5 to 0.6. The same value is found by following the amplitude of the rapid fluorescence relaxation signal after preincubation with 220 nM Tpo. The present results also show that, in parallel with the shift of the R = D equilibrium, Tpo causes an increase of the rate of the intermediate relaxation process.

When Ca\textsuperscript{2+} was omitted or replaced by Mg\textsuperscript{2+}, all the allosteric effects observed in vitro at low concentrations of Tpo disappeared. Single-channel experiments with C\textsubscript{2} mouse myotubes yielded similar findings. Indeed, cell-attached recordings performed in a Ca\textsuperscript{2+}-free extracellular medium failed to demonstrate any effect of low Tpo concentrations on AcChoR desensitization and only moderate effects when higher concentrations of Tpo were used. Moreover, outside-out recordings further revealed that (i) rapid chelation of Ca\textsuperscript{2+} entry by high concentrations of EGTA (10 mM) prevented the occurrence of the shifts and (ii) Tpo was effective when internal Ca\textsuperscript{2+} was allowed to fluctuate in the presence of low concentrations of EGTA. Thus, the concentration of Ca\textsuperscript{2+} present inside the cell regulates the effect of Tpo on desensitization. In other words, the conjunction of Tpo and Ca\textsuperscript{2+} on the opposite faces of the membrane is required for the enhanced desensitization of the AcChoR by Tpo. A similar mechanism has been postulated, on strictly theoretical grounds, to model changes of synapse efficacy that potentially occur in physiological situations referred to as "classical conditioning" (2).

The nature of the site(s) at which Tpo enhances AcChoR desensitization is (are) as yet unknown. The difference in the response to Tpo could be attributed to the occupancy of AcCho binding sites and for the regulation of desensitization suggests that high affinity allosteric sites for Tpo distinct from the AcCho binding site may exist on the AcChoR-rich membranes. These sites remain after the membranes are depleted of peripheral proteins by treatment at pH 11 (20). Such sites, however, were not detected in previous studies (10) where cholinergic ligands were found to totally inhibit Tpo binding to AcChoR-rich membranes. Alternatively, since the two AcCho sites present per AcChoR oligomer possess different binding properties (for review, see ref. 1), Tpo might bind preferentially to one of these sites thus stimulating desensitization while leaving the other one accessible to Dns-C\textsubscript{2}Cho or AcCho.

Alternative mechanisms for the desensitizing effect of Tpo could be considered. However, no phospholipase A or C activity (23) could be detected in our Tpo preparation (24). Moreover, the allosteric effects of Tpo do not seem to be linked to phosphorylation (25, 26), since T. marmorata membrane preparations and cell membranes in the outside-out configuration presumably do not contain the full complement of factors required for phosphorylation.

The present data could explain previous physiological studies suggesting that a hormone secreted by the thymus (Tpo) affects cholinergic transmission at the neuromuscular junction (9). The level of biologically active Tpo measured by immunoassays (27) in bovine serum varies from 30 to 50 nM, a range of concentration within which Tpo may regulate AcChoR desensitization. Changes in serum Tpo occasioned by thyectomy or implantation of thymus grafts could, therefore, account for the changes in cholinergic transmission observed in thymetomized or thymus-grafted rats (28).

The pathogenesis of the neuromuscular block in myasthenia gravis is controversial. Autoantibodies to the AcChoR are often present, but the lack of systematic correlation between the levels of such antibodies and the state of neuromuscular transmission (29) suggest that these autoantibodies may not represent the only pathogenic mechanism involved in the disease. The present data lend further support to the hypothesis (4) that an autoimmune thymus results in the elevation of circulating Tpo levels could lead to depression of cholinergic transmission at the neuromuscular junction. They further suggest that the thymus, by means of Tpo, in addition to its known actions on the immune system, could exert regulatory effects at the neuromuscular junction.

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