tion; the mitochondria of the muscles may be stimulated more slowly than the hepatic mitochondria.

Mitochondria from many of the tissues possess saturable receptors—not only hepatic mitochondria but also mitochondria from the kidney, myocardium (atrial and ventricular), skeletal muscle, lung, small intestine, adipose tissue, and the brains of neonates (*2*). In contrast, no such receptors have been found in mitochondria from adult brain, spleen, or testes, organs that are calorigenically unresponsive to thyroid hormone (*14*).

Summary. It is probable that there is no single intracellular effector for thyroid hormone action; rather, several pathways may combine to give an integrated response. Early effects that are independent of protein synthesis have been described at the level of the plasma membrane (*15-23*). Binding by nuclear chromatin may be related to anabolic effects not evident until after a lag period; such effects may be essential to normal growth, differentiation, and cell maintenance, but are probably sustained, delayed effects rather than immediate or initiating actions. Activation of mitochondrial energy metabolism seems a likely mechanism behind the earliest effects, such as increased heart rate and oxygen consumption a few hours after intravenous injection of T_3 in myxedema patients (*24*).

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Lithium Reduces the Number of Acetylcholine Receptors in Skeletal Muscle

Abstract. *Extended treatment of rats with lithium inhibits the increase in the number of extrajunctional acetylcholine receptors that occurs in their denervated skeletal muscle. In normal muscle, lithium reduces the number of acetylcholine receptors at neuromuscular junctions. These changes appear to be a relatively specific effect of lithium on the turnover of receptors. Skeletal muscle provides an accessible system for analyzing the role of lithium (and other cations) in the regulation of cell surface receptors. This regulation may play a role in the mechanism by which lithium prevents recurrent manic-depressive episodes.*

The distribution and turnover of acetylcholine (ACh) receptors in mammalian skeletal muscles are regulated by intrinsic muscle cell mechanisms, which are largely influenced by motor nerves (*1*). In innervated muscles, most ACh receptors are located at the neuromuscular junctions, but after denervation (*1*) or pharmacological blockade of cholinergic transmission (*2*) there is a great increase in the density of extrajunctional ACh receptors. In the central nervous system, receptors for various neurotransmitters appear to be similarly regulated. For example, after surgical lesions of the nigrostriatal dopamine pathway (*3*) or pharmacological blockade of dopamine transmission (*4*), supersensitivity to dopamine and an increase in the number of dopamine receptor sites have been observed. It was recently reported that lithium treatment prevents these denervation-like changes occurring after blockade of dopamine transmission (*5*). The present study was undertaken to determine whether lithium alters the denervation-induced increase in the number of extrajunctional ACh receptors in skeletal muscle.

We injected 0.4*M* LiCl (1.0 ml, intraperitoneally) twice daily into female Sprague-Dawley rats weighing 230 to 270 g. Control animals were given injections of equal amounts of 0.4*M* NaCl (1.0 ml). After 1 to 2 days, the soleus muscle was denervated by avulsing the sciatic nerve in the thigh under cloral hydrate anesthesia (400 mg/kg). At 4 and 7 days after denervation, the soleus muscles were removed and ACh receptors were measured by a method that utilizes saturation binding of [^{125}I]α-bungarotoxin (α-BuTx) (*6*). The specific and irreversible binding of this snake venom fraction to ACh receptors allows quantitative measurement of the receptors (*7*). Junctional ACh receptors were measured in innervated muscles and extrajunctional ACh receptors were measured in both denervated and innervated muscles. The muscle fiber diameters were measured in soleus muscles pinned at resting length and quickly frozen in isopentane cooled with solid CO_2. Transverse sections 6 μm thick were cut in a crytostat and stained with hematoxylin and eosin, modified Gomori's trichrome, and adenosine triphosphatase (*p*H 4.3 and 9.4). A microscope with an ocular micrometer was used to measure the muscle fiber diameters; mean diameters were computed from at least 50 fibers in each muscle.

Lithium treatment markedly inhibited the increase in the number of extrajunctional ACh receptors in the denervated muscles. Seven days after denervation, the mean density of extrajunctional ACh receptors in the soleus muscles of lithium-treated animals was only 39 percent as high as that in saline-treated controls (Table 1) ($P < .001$). Similarly, 4 days after denervation, the density of extrajunctional ACh receptors in soleus muscles from the lithium-treated animals (70 ± 17 per square micrometer) was only 39 percent of the value for the saline-treated animals (185 ± 48) ($P < .02$). Lithium treatment also produced a significant ($P < .005$)

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decrease in the number of ACh receptors at neuromuscular junctions of innervated muscles (Table 1).

The method of intraperitoneal injection of lithium used produced serum levels of lithium fluctuating from $1.5 \times 10^{-3}M$ (therapeutic for humans) 1/2 hour after injection to $0.2 \times 10^{-3}M$ 6 hours after injection. The reduction in the number of ACh receptors may have been even more pronounced had therapeutic levels of lithium been maintained throughout the experiment. Lithium-treated rats showed typical pharmacological effects, including polydipsia and polyuria, within 2 to 3 days. There were no deaths in the experimental group, and only 2 of 23 rats showed signs of lithium toxicity (weight loss, decreased drinking, and irritability); these animals were discarded. Histological and histochemical examination of muscles from the lithium-treated rats revealed no evidence of muscle degeneration or other pathological changes. Seven days after denervation, the mean diameter of soleus muscle fibers from the lithium-treated rats was significantly larger than that of fibers from the saline-treated animals.

The mechanism by which lithium reduces the number of ACh receptors is not known. This effect cannot be attributed to an overall change in muscle metabolism resulting in a nonspecific loss of protein. First, the mean diameter of muscle fibers was actually larger in lithium-treated animals than in controls. Furthermore, we have found that extended treatment of skeletal muscle cultures with lithium does not reduce the amount of total protein, incorporation of [^{3}H]leucine into protein, or the activity of creatine phosphokinase, a muscle-specific protein (*8*). Also, exposure to lithium does not interfere with the ability of ACh receptors to bind α-BuTx, since the addition of LiCl ($1.5 \times 10^{-3}M$) to cultured rat myotubes did not reduce the binding of ^{125}I-labeled α-BuTx. Although lithium was reported to alter neurotransmitter release (*9*), this cannot be its mechanism of action in surgically denervated muscles. Therefore it is likely that lithium acts at the muscle cell level to influence the turnover of ACh receptors. Since lithium causes a decrease in the number of ACh receptors, receptor synthesis and incorporation into the surface membrane must be reduced or degradation must be increased, or both must occur. Preliminary studies suggest that lithium selectively increases the rate of degradation of ACh receptors without affecting their synthesis (*8*).

Table 1. Effect of lithium on normal soleus muscles and on muscles 7 days after denervation. Lithium treatment extended over 9 days. Data are expressed as means ± standard errors; numbers in parentheses are the number of muscles sampled.

Group	Extrajunctional ACh receptors per square micrometer	ACh receptors per neuromuscular junction ($\times 10^7$)	Fiber diameter (μm)
Innervated			
Lithium-treated	15 ± 2 (7)	1.8 ± 0.2 (17)*	48 ± 1 (6)
Control	12 ± 3 (9)	2.8 ± 0.3 (16)	49 ± 1 (6)
Denervated			
Lithium-treated	105 ± 29 (8)†		37 ± 1 (6)‡
Control	269 ± 27 (8)		33 ± 1 (6)

*Different from controls at $P < .005$. †Different from controls at $P < .001$. ‡Different from controls at $P < .05$.

How lithium influences the turnover of ACh receptors remains a matter for speculation. The synthesis of certain other specific cell proteins is regulated by concentrations of intracellular cations (*10*). Since lithium shares important properties with each of the more abundant intracellular cations Na^+, K^+, Ca^{2+}, and Mg^{2+} (*11*), it is tempting to speculate that its effect on ACh receptor metabolism may be related in some way to its properties as a cation. Future studies of the effects of lithium on ACh receptor metabolism may lead to better understanding of the role of cations in the regulation of surface receptors in muscle, brain, and other cell systems.

It has been suggested (*5*) that alterations in catecholine receptor sensitivity in the brain may account for the behavioral abnormalities in manic-depressive disorders. It has now been shown that lithium can reduce the number of acetylcholine receptors in muscle and the number of dopamine receptors in the brain. Although the effects of lithium may be complex, these and other experiments suggest that the ability of lithium to prevent recurrent manic-depressive disorders may be related, at least in part, to an ability to reduce the number of cell surface receptors.

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