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19. High pressure liquid chromatography (HPLC) was used as a second identity validation for melatonin. Ten 1-ml samples of alligator plasma, one containing [$^3$H]melatonin (5000 count/min; New England Nuclear), were extracted with chloroform, and washed in sodium bicarbonate buffer (*p*H 10.25) and distilled water. The samples were concentrated and dried with $N_2$ gas. The dried extract was resuspended in 250 $\mu$l of chloroform and filtered through a 0.45-$\mu$m Teflon filter. The filtrate was injected onto a Waters $\mu$Bondapak $NH_2$ HPLC column (30 cm) with a 10-minute linear solvent gradient (50 percent tetrahydrofuran and 50 percent isooctane to 100 percent acetonitrile containing 1 percent acetic acid). Every 15 seconds, 0.5-ml fractions were collected, dried with $N_2$ gas, and resuspended in 350 $\mu$l of phosphate-buffered saline gel buffer; the $^3$H was determined by liquid scintillation in each resuspended 100-$\mu$l portion. Two 100-$\mu$l portions were analyzed by RIA for melatonin (Fig. 1). [$^3$H]Melatonin is present in larger amounts in fraction 31 (7:30 to 7:45 minutes) or fraction 32 (7:45 to 8:00 minutes).
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## Neuronal Control of Acetylcholine Receptor Turnover Rate at a Vertebrate Neuromuscular Junction

Abstract. *The turnover rate of acetylcholine receptors at neuromuscular junctions in mice increases progressively after denervation and, after 15 days, reaches a half-time of 30 ± 5 hours. Denervation thus causes the clustered junctional acetylcholine receptors to assume the rapid turnover characteristic of extrajunctional receptors before innervation.*

It is well documented that nerves influence the metabolism and physiology of skeletal muscle (*1–3*), although the mechanisms behind this are unknown. One expression of neuronal control is the progressive decrease in the turnover of acetylcholine (ACh) receptors which occurs at the neuromuscular junction (NMJ) after innervation (*4–6*). Two distinct turnover rates for ACh receptors in muscle have been described: for extrajunctional receptors (before innervation and after denervation), a half-time of about 1 day (*6–11*), and for adult innervated junctional receptors, a half-time of about 10 days (*7–16*). Recently, however, a half-time of 2 to 3 days for denervated adult junctional ACh receptors was reported (*12–13*). In the present study we sought to determine whether the denervated junctional ACh receptors demonstrate a third stable turnover rate or whether the reported half-time of 2 to 3 days represents a point in a time-dependent decay of neuronal influence on the turnover rate of junctional ACh receptors after denervation.

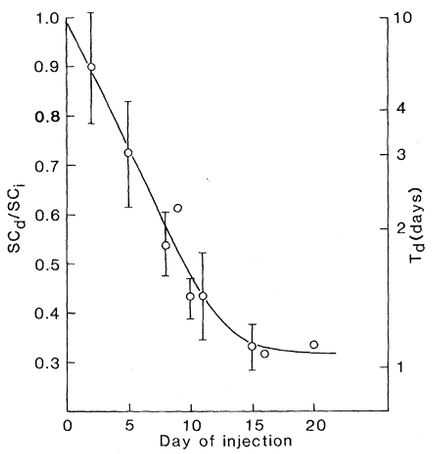


Fig. 1. Ratio of residual radioactivity at denervated ($SC_d$) and innervated ($SC_i$) NMJ's of mice injected with $^{125}$I-labeled $\alpha$-BGT at various times after denervation and killed 2 days ($t$) later. The average half-time for turnover of denervated junctional receptors ($T_d$) was approximated from the ratio of the two exponential decays, $SC_d/SC_i = (2 \exp - t/T_d)\, SC_{d_0}/(2 \exp - t/T_i)SC_{i_0}$, with an average half-time for turnover at innervated junctions of 10 days (*11*) and given that the radioactivity in the two junctions at time zero is the same $[(SC_{i_0}) = (SC_{d_0})]$. Errors bars represent standard errors of the means for groups of three to seven animals. Data points without error bars represent values for single animals.

We assessed the turnover of ACh receptors by measuring the loss of radioactivity bound to the NMJ's after an injection of $^{125}$I-labeled $\alpha$-bungarotoxin ($\alpha$-BGT), the inhibitor of the nicotinic ACh receptor of vertebrate muscle (*17*). This assay gives a reliable measure of the degradation of both extrajunctional and junctional ACh receptors (*1, 9, 14–16, 18–20*) rather than of mere dissociation of $\alpha$-BGT from the receptors. For the innervated NMJ's the validity of this assay obtains in part from the demonstration that various metabolic inhibitors cause a decrease in the rate of both the loss of radioactivity after labeling (*9, 14–15*) and the recovery of neuromuscular response (*21*) after inactivation with $\alpha$-BGT. In preliminary studies we found that actinomycin D similarly decreases the rate of loss of radioactivity from denervated NMJ's. Furthermore, up to 16 days after denervation, structural localization and absolute density of radioactive sites after inactivation at the NMJ's by $^{125}$I-labeled $\alpha$-BGT is the same as that at innervated junctions, and at both kinds of junctions the loss of radioactivity mirrors the appearance of new labeled binding sites (*13*). Because of this steady state at the NMJ, degradation reflects metabolic turnover of receptors.

We used mouse sternomastoid muscle since it has an easily accessible nerve and a well-defined end-plate band and was previously used in our laboratory for electron microscope (EM) autoradiography studies of receptor localization (*20*) and turnover after denervation (*13*). We anesthetized the mice (27 to 37 g) with Nembutal and denervated one of the two sternomastoid muscles by removing 1 to 2 mm of nerve as close to the muscle as possible.

At different times after denervation, groups of these animals were injected with $^{125}$I-labeled $\alpha$-BGT (4.1 $\mu$g per 100 g of body weight, intraperitoneally) (*22*). Two days later, the animals were killed under anesthesia by intracardial perfusion with 4 percent paraformaldehyde and the two sternomastoid muscles were removed and stained for acetylcholinesterase in order to identify the end-plate

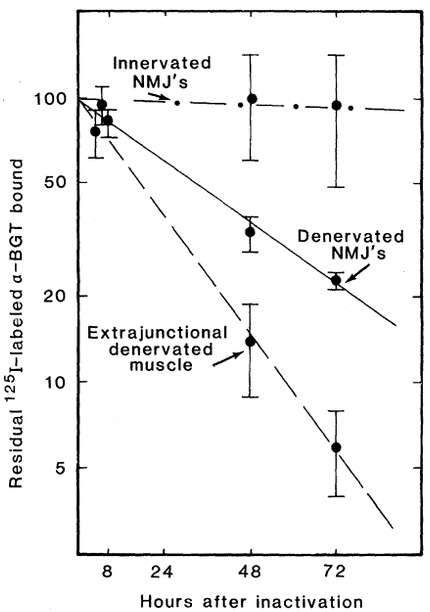


Fig. 2. Decay of radioactivity bound to innervated and denervated sternomastoid muscles after injection of labeled $\alpha$-BGT. All curves are normalized to 100 percent at the time of injection.


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band (*23*). The muscles were cut into three pieces (one containing the stained end plates), and the pieces were weighed and assayed for radioactivity in a gamma counter. Specific end-plate radioactivity (*SC*) was determined for each muscle by subtracting the pooled radioactivity bound to the pieces without end plates from the piece containing the end plates on a per weight basis. A ratio of the specific counts for denervated to innervated junctions was determined for each animal; the average at each time is presented in Fig. 1. We have determined that there is no significant difference between the amount of radioactivity bound to the left and right muscles under control conditions. Furthermore, it has been established (*13*) that the turnover rate for junctional ACh receptors determined by the gamma counting method is very close to that determined by EM autoradiography for the radioactivity at the postjunctional membrane.

Figure 1 shows that after denervation the radioactivity progressively decays more rapidly at denervated junctions than at innervated junctions. Eight days after denervation the half-time for turnover of denervated junctional receptors was 2 ± 0.4 days, which is consistent with the value derived from EM autoradiography (approximately 2.5 days) (*13*).

An independent comparison of the turnover rates of denervated extrajunctional and junctional receptors with those of normal junctional receptors was also made. Fourteen animals were injected with $^{125}$I-labeled α-BGT 11 days after denervation. Four, seven, and three mice were killed 8 hours, 2 days, and 3 days after the injections, respectively, and the muscles were removed. The specific end-plate counts for denervated and innervated muscles and the extrajunctional radioactivity were each normalized to 100 percent at time zero and plotted semilogarithmically (Fig. 2). These data show the exponential nature of the decay for the denervated junctions over this 3-day period.

Eleven days after denervation the half-time for denervated junctional receptors was 34 ± 5 hours (Fig. 1), and the independently derived value was 33 ± 8 hours (Fig. 2). Fifteen days after denervation, the half-time for turnover at denervated junctions reached 30 ± 5 hours. By that time, the turnover rate for junctional ACh receptors was well within the range of values reported (*7, 8*) for extrajunctional receptors in noninnervated muscle and approaching the value of 17.5 hours given in Fig. 2 (*24*).

It has been reported that a maturation period is required between the time that clusters of ACh receptors first appear at the forming NMJ's and the time that the turnover rate of these junctional receptors decreases (*4-6, 25*). The present data suggest that there is a finite period after denervation during which the neuronal influence on turnover of junctional ACh receptors fades, even though the dense clustering remains unchanged (*13*). Thus the acceleration of turnover may reflect a reversal of the process of maturation of junctional receptors seen during development.

*Note added in proof*: Since this report was accepted for publication, we have obtained evidence that Fig. 1 can be modeled by assuming that the junction contains a dual population of receptors: the original receptors, present at the time of denervation, with a slowly increasing turnover rate, and new ones, with a turnover rate equivalent to that of extrajunctional receptors (*26*).

TAMI A. LEVITT
RALPH H. LORING*
MIRIAM M. SALPETER
*Section of Neurobiology and Behavior* and *School of Applied and Engineering Physics, Cornell University, Ithaca, New York 14853*

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* Present address: Department of Pharmacology, Harvard Medical School, Boston, Mass. 02115.

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## Maternal Glucocorticoid Hormones Influence Neurotransmitter Phenotypic Expression in Embryos

Abstract. *Treatment of pregnant rats with reserpine prevented the normal disappearance of catecholamine fluorescence in presumptive neuroblasts of the embryonic gut. These cells normally express the noradrenergic phenotype transiently during embryonic development. The effect of reserpine was reproduced by treating mothers with hydrocortisone acetate. Moreover, the reserpine effect was blocked by treatment with dexamethasone, which inhibits the stress-induced increase in plasma glucocorticoids, and by mitotone, which causes adrenocortical cytolysis. It is concluded that reserpine, through the mediation of maternal glucocorticoid hormones, alters the phenotypic expression of these embryonic neuroblasts.*

Although it is recognized that maternal-fetal relations critically influence the developing nervous system, the processes by which maternal experience affects embryonic development are ill-defined. Exposure of pregnant or nursing animals to antipsychotic agents (*1*), opiates (*2*), or reserpine (*3*) is associated with neurological and behavioral abnormalities in the offspring. Moreover, maternal conditions such as toxemia, advanced age (*4*), and stress (*5*) also contribute to neurological defects.

The current study was undertaken to define the effects of neurologically active drugs on the embryonic nervous system. Specifically, we sought to characterize the influence of pharmacologic agents on


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