NOTES AND NEW DEVELOPMENTS

PREDNISONE-NEOSTIGMINE INTERACTIONS AT CHOLINERGIC JUNCTIONS

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Abstract: The effect of corticosteroid (prednisone) and/or chronic anticholinesterase (neostigmine) treatment on α-bungarotoxin binding was examined in the diaphragms of male rats. In endplate regions of the diaphragm, prednisone treatment had no effect on the density of toxin binding sites, either when given alone or when administered in conjunction with neostigmine, while neostigmine was observed to reduce specific binding to less than half after one week.

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Chronic treatment of rats with the anticholinesterase neostigmine is associated with reduced acetylcholine (ACh) release, decreased amplitude of synaptic potentials,[7] simplification of endplate ultrastructure,[3,8] and decreased binding of α-bungarotoxin at the neuromuscular junction.[2] All of these changes would be expected to interfere with neuromuscular transmission. Similar disturbances are found in myasthenic patients, many of whom have been on long-term anticholinesterase therapy. Although it is possible that some of these clinically observed alterations are due to the anticholinesterase treatment, similar abnormalities are found in untreated animals with experimental autoimmune myasthenia gravis (EAMG). Nevertheless, neostigmine also causes marked improvement of neuromuscular transmission both in human myasthenia gravis and in the experimental animal model of the disorder, indicating that the net pharmacologic effect represents interaction of several opposing factors. A reduction in the number or density of postjunctional ACh receptors would, however, be particularly harmful to the myasthenic patient, and thus it seemed appropriate to study mechanisms that might control the detrimental actions of neostigmine while leaving its therapeutic effects unchanged. In particular, we wished to investigate whether the well-known efficacy of prednisone treatment in myasthenia gravis might result, in part, from an ability of the hormone to preserve receptor sites from inactivation or destruction by cholinesterase inhibitors. We therefore used ^{125}I-α-bungarotoxin (α-BuTx) as a probe to test changes in numbers of binding sites, comparing results obtained with prednisone alone to those obtained with prednisone and neostigmine.

MATERIALS AND METHODS

Male Wistar rats weighing 150–225 g were injected at the base of the tail with 0.1 mg of neostigmine methylsulfate (Roche Laboratories) twice a day for seven days, and/or with 5 mg of methylprednisone acetate (Upjohn Company) on the first and third days. To reduce muscarinic side effects, 1 mg of atropine sulfate (USP) was given 30

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min before each neostigmine dose for the first two days. Seven days after the initial injection, animals were sacrificed and diaphragms were removed. Tissues were mounted in a small Petri dish containing a layer of Paraplast (Sherwood Laboratories), after which they were washed for 1 hr (three changes of medium).

Fresh medium was then added in sufficient quantity to cover the tissue, followed by ^{125}I-α-BuTx to reach a concentration of 0.5 μg/ml, which was shown to saturate toxin binding in these preparations. Tissue was incubated with shaking at 20°C for 75 min and was then washed with changes of fresh medium until the radioactivity level in the wash medium was no more than 20% above background.

After washing, diaphragms were divided into endplate and nonendplate regions. The endplate regions and portions of the nonendplate regions were cut into several pieces and weighed; they were then digested overnight in 0.3 ml of 0.5 N KOH prior to counting in a Nuclear Chicago deep-well gamma counter.

α-BuTx was purified from crude venom (obtained from the Miami Serpentarium) by the method of Lee et al[5] and was iodinated by a modification of the method of Marcholonis.[6] Next, 20–50 μg of α-BuTx (5 mg/ml in 0.1 M potassium phosphate buffer, pH 7.4) was added to 20 μl of carrier-free Na^{125}I (Amersham-Searle, 100 mCi/ml). Lactoperoxidase was added every 20 min for 1 hr to reach a final concentration equal to 0.5 mole per mole of α-BuTx. Hydrogen peroxide was added every 15 min for 1 hr to reach a final concentration of 100 μM. Reaction volumes ranged from 30 to 50 μl, reaction efficiencies from 70% to 90%. Specific activities ranged from 6.4×10^4 to 8.5×10^5 Ci/mole. The potency of each batch of radiolabeled α-BuTx was tested prior to use by recording its ability to reduce or eliminate the indirect twitch response in the phrenic nerve–diaphragm preparation,[2] or by assay of specific binding to test diaphragms.[1] The one-tailed Student's t test was used for all statistical comparisons.

RESULTS

Prednisone. Prednisone treatment caused a 12% reduction in body weight and an 18% reduction in hemidiaphragm weight ($p < 0.005$) (table 1). In stained preparations, no gross changes were observed in cell size or in architecture, and the weight reductions accompanying prednisone treatment may have been due to a decrease in body fat as a result of the anorexia present in prednisone-treated animals. Despite the weight loss, there was no evidence of reduction in the total number of toxin binding sites in the endplate region, because the ratio of counts per minute (cpm) per milligram of treated to control tissue increased almost exactly as the ratio of control to treated animal hemidiaphragm weights (1/0.82 = 1.22). However, toxin binding in the nonendplate regions increased significantly (126.3%, $p < 0.05$) with prednisone treatment.

Prednisone plus Neostigmine. As reported by Chang et al,[2] chronic neostigmine administration also caused a significant reduction in body weight (18%), in the weight of the hemidiaphragms (35%), and in ^{125}I-α-BuTx binding capacity (45%) of the endplate regions of the hemidiaphragm preparation. Given together, prednisone and neostigmine caused a more severe reduction in whole body weight than did neostigmine alone. The administration of prednisone neither aggravated nor alleviated the

Table 1. Data for α-bungarotoxin bound, tissue weight, and whole body weight. The data are given as the ratios of values for animals in the different groups.

	Experimental group[a]		
	Prednisone + neostigmine / Neostigmine	Prednisone / Control	Neostigmine / Control
Toxin bound to hemidiaphragm endplate region (Fmoles/mg tissue)	1.05 (9/9)	1.02 (14/14)	0.45 (10/10)[b]
Hemidiaphragm weight (g)	0.90[b] (9/9)	0.82 (14/14)[b]	0.65 (10/10)[b]
Body weight (g)	0.76[b] (5/5)	0.88 (14/14)[b]	0.82 (10/10)[b]

[a] *Values in parentheses indicate numbers of animals in the groups being compared.*
[b] $p < 0.005$

neostigmine-induced alterations in ^{125}I-α-BuTx binding to the endplate region of the hemidiaphragm (table 1).

DISCUSSION

Myasthenic patients often receive corticosteroids in conjunction with anticholinesterase medication, and the results are often better than those achieved with neostigmine alone, although prednisone has not been shown to have a direct facilitatory action on the endplate.[4] The question of interest is whether the observed clinical benefit represents a pharmacologic action at the neuromuscular junction or an indirect effect on the neostigmine response. Specifically, could prednisone reduce the magnitude of the neostigmine-induced reductions in ^{125}I-α-BuTx binding?

The data clearly show that large doses of prednisone cause significant weight loss in whole animals and in the diaphragm. When both prednisone and neostigmine are given together, the general weight loss is still greater. After toxin binding is normalized with respect to changes in diaphragm weight, the following conclusions may be made: (1) large doses of prednisone do not increase the number of junctional receptors in the normal animal; (2) the same doses, however, cause an increase in α-BuTx binding sites away from the endplate; and (3) prednisone does not inhibit the neostigmine-induced changes in endplate and nonendplate receptor density. It seems likely, therefore, that corticosteroid compounds exert their potent antimyasthenic action through other parameters of neuromuscular or immunologic function.

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