Corticosteroids and Neuromuscular Transmission:
Electrophysiological Investigation of the Effects of Prednisolone
on Normal and Anticholinesterase-Treated Neuromuscular Junction

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Abstract. The effect of prednisolone on indirectly stimulated rat muscle twitch was investigated at normal and prostigmine-treated neuromuscular junctions. In vivo, prednisolone up to 150 mg/kg body weight did not affect twitch contraction in normal animals. In neostigmine-pretreated animals, however, doses between 12.5 and 90 mg/kg could entirely abolish the anticholinesterase-induced twitch augmentation. In vitro, prednisolone produced a depressant effect on the twitch of a normal phrenic nerve diaphragm preparation which could amount to 20%. When the preparation was pretreated with neostigmine the augmented twitch could be depressed by 10^{-3} to 10^{-6} mol/l prednisolone to levels below the untreated control. Part of this effect is owing to a suppression of the neostigmine-induced, stimulus-bound repetitive firing of the motor nerve terminals, but to explain the full effect a further inhibitory action on neuromuscular transmission must be assumed. The latter could be accounted for by a depolarizing interaction of prednisolone and neostigmine on the nerve terminals resulting in conduction block. An action of prednisolone on postsynaptic receptors could also be considered. Such effects of the glucocorticoid might contribute to the exacerbation of muscular weakness occasionally observed in patients with myasthenia gravis at the beginning of steroid therapy.

Key words: Neuromuscular transmission — Repetitive nerve firing — Anticholinesterases — Corticosteroids — Myasthenia gravis.

Introduction

Possible direct effects of adrenal corticosteroids on neuromuscular junctions have been the subject of several recent investigations [1—4, 8, 11, 14, 17, 20, 21]. The steroids seem to affect neuromuscular transmission in a complicated way so that some investigators suggested the effects would be facilitatory [1, 11, 17, 21], others to be inhibitory [8, 14] to neuromuscular transmission. Baker et al. [2] reported a biphasic direct action of methylprednisolone on facilitation of neuromuscular transmission consisting of an immediate suppression and an enhancement 18—24 h later.

Since corticosteroids have been of growing importance for the treatment of myasthenia gravis [6, 19], a thorough knowledge of their direct effects on the neuromuscular junction seems desirable, in particular, because a transient exacerbation of weakness has occasionally been observed at the beginning of steroid therapy [6]. Such a depression of function suggests an immediate pharmacological inhibition which predetermines before the delayed immunosuppressive effect can develop.

Patten et al. [14] have reported adverse effects of steroids on neuromuscular transmission when given in conjunction with cholinesterase inhibitors. As an explanation of these findings, they discussed a possible effect of the steroids on the repetitive activity induced in the motor neurons by cholinesterase inhibitors. The latter are known not only to improve neuromuscular transmission in endplates which are partially blocked, e.g. by curare, but also to augment the twitch contraction of a nerve-muscle preparation which has not been blocked. This augmentation has first been described by Masland and Wigton [12]. It is caused by a series of afterdischarges in the motor nerve following a stimulated action potential. Careful tests which have been reviewed by Riker and Okamoto [16] have excluded extra- or intrafusal muscle fibres as the source of the afterdischarges. It is therefore believed that the repetitive firing is generated in the motor nerve terminals from where it is conducted to additional axon terminals.

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within the same motor unit thus augmenting contraction [7, 13, 16]. Such an augmented "twitch", then, is in fact a kind of asynchronous tetanus.

Since a combination of cholinesterase inhibitor and corticosteroid is common in the treatment of myasthenic patients we felt it necessary to confirm Patton's experiments and to test their hypothesis. The results have already been reported to the German Physiological Society.

**Material and Methods**

*In vivo Experiments.* Male Wistar rats (350 – 500 g) were anesthetized by i.m. injection of 50 mg/kg ketamine hydrochloride (Ketanest, Parke Davis, München) and 5.7 mg/kg xylazine hydrochloride (Rompun, Bayer, Leverkusen). A cannula was inserted into the right jugular vein and a tracheal catheter was placed to prevent respiratory inhibitor for a short time only. By trial we found that under our conditions 45 s of neostigmine methylsulphate dose of 0.0125 mg/kg. Since a combination of cholinesterase inhibitor and neostigmine doses or longer perfusion produced an undesired depression of force following a short period of augmented contractions. In fact, in 5 out of 15 experiments described below even the 45 s of neostigmine exposure produced signs of this depression. In these cases we observed a biphasic time course consisting of an initial rise in force and a brief fall before twitch force settled at a stable augmented level (see Figs.2B and 5).

Prednisolone was applied in concentrations of $10^{-2}$, $10^{-4}$, $10^{-6}$ and $10^{-7}$ mol/l. Because of the above mentioned instability of neostigmine-induced augmentation of contraction, two types of experiments were performed. In 15 experiments, later referred to as type B, prednisolone was given at a variable time after the 45 s application of neostigmine (see Figs.2B and 5). Values obtained immediately before and 3 – 7 min after prednisolone perfusion were compared. In 6 experiments, later referred to as type C, prednisolone was applied immediately after the 45 s of neostigmine administration (see Fig.2C). Values taken 7 min after prednisolone application were compared with corresponding data of control experiments, where neostigmine was followed by drug-free solution.

Nerve action potentials and muscle contractions were recorded on an oscilloscope and photographed. To quantify repetitive firing the filmed records were enlarged and the number of spikes during the first 50 ms following a stimulated compound action potential were counted by eye. Sometimes the early phase of stimulus-bound repetitive firing occurred at such high rate that spikes were superimposed or fused. In this case quantification became less accurate.

In order to treat the results statistically, for each experiment the maxima of contraction amplitude, time to peak of contraction and of spike rate induced neostigmine were taken as 100% and the percentage of corresponding values obtained in the control phase, and before and after prednisolone application were calculated. Means ± S.D. of these percentages were calculated separately for each prednisolone concentration in type B experiments, in type C experiments data from different prednisolone concentrations were pooled. Since Student's t-test showed significant differences when comparing the percentages of spike rates counted before and after prednisolone application, no further steps were taken to improve the above mentioned inaccuracy of spike counting.

**Results**

*In vivo-Experiments*

In vivo-experiments were carried out as pilot studies in order to test the range of the effective dosages of neostigmine and prednisolone. In 2 otherwise untreated animals successive doses of prednisolone to a total of 150 mg/kg over 10 min did not affect twitch amplitude.

In 9 animals neostigmine methylsulphate, 0.0125 – 0.125 mg/kg, regularly increased the contraction response on a single stimulus to 150 – 300% within 2 – 3 min. Injection of prednisolone following neostigmine pre-treatment abolished this augmentation in part or entirely. The lowest effective dose of prednisolone (12.5 mg/kg) showed this depressant action only if augmented contraction had been produced by the low neostigmine methylsulphate dose of 0.0125 mg/kg. After a higher neostigmine dose also a higher prednisolone dose was necessary to produce a depression of the twitch. For instance, in the experiment of Fig.1 the neostigmine methylsulphate dose of 0.025 mg/kg caused an augmentation of contraction to 300% within