EFFECT OF REPEATED ADMINISTRATIONS OF NEOMYCIN ON THE NEUROMUSCULAR FUNCTIONS AND THE ENHANCEMENT OF *D*-TUBOCURARINE ACTION IN MICE

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ABSTRACT — Effects of repeated s.c. treatments with 50 μ mol (approx. 31 mg)/kg/day of neomycin for 10 or 15 days were examined in mice. There were no significant differences between saline and neomycin treatment groups in the motor coordination assessed by rota-rod test and traction test. On tension recordings, an *in vitro* addition of *d*-tubocurarine inhibited the twitch tensions evoked by the nerve stimulations in both cases of saline or neomycin treatment. Neomycin treatments shifted the concentration-response curve between twitch tension and *d*-tubocurarine to the left, dependently on its injection days. These results suggest that repeated treatments with neomycin enhance the inhibitory effect of *d*-tubocurarine on the neuromuscular transmission in mice without eliciting motor incoordination and muscle relaxation.

KEY WORDS : Neomycin, Repeated treatments, Rota-rod test, Neuromuscular twitch, *d*-Tubocurarine.

INTRODUCTION

Neomycin is an aminoglycoside antibiotic, discovered from a strain of *streptomyces* (Waksman and Lechevalier, 1949). Since this antibiotic shows broad antibacterial spectrum, it has been tried to treat gram-negative infections (Pulaski *et al.*, 1950 ; Livingood *et al.*, 1952 ; Poth, 1952). However, aminoglycoside antibiotics are apt to possess neuromuscular blocking action as a side effect and neomycin has the most potent action (Pittinger and Adamson, 1972). The combination of neomycin with anesthetic agents causes an acute apnea by blocking respiratory muscles (Pridgen, 1956). *In vitro*, neomycin

reduces voltage-dependent Ca^{2+} -influx into nerve terminal at neuromuscular junctions (Wright and Collier, 1977) and synaptosome (Atchison *et al.*, 1988), and decreases Ca^{2+} -dependent acetylcholine (ACh) release from motor nerve terminals (Elmqvist and Josefsson, 1962 ; Vital Brazil and Prado-Franceschi, 1969 ; Fiekers, 1983).

Durant and co-workers (1981) reported that neomycin increases in the sensitivity of neuromuscular transmission to *d*-tubocurarine despite apparently normal responses to indirect stimulation in anesthetized cats. However, it is unknown whether the continual inhibition of ACh release and neuromuscular transmission by repeated pretreatments with neomycin *in vivo* affects the action of *d*-tubocurarine and anesthetic agents in surgical operation or not. Thus, we investigated to reveal the effects of repeated pretreat-

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ments with neomycin on the motor coordination assessed by rota-rod test and traction test, on the twitch tension of diaphragm evoked by nerve stimulation and on the sensitivity to *d*-tubocurarine, a competitive blocker of nicotinic ACh receptor.

MATERIALS AND METHODS

Drugs: Neomycin sulfate and *d*-tubocurarine chloride were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals used were analytical grade.

Animals and treatments with neomycin: Male *ddY* mice (purchased from SLC, Shizuoka, Japan) were used in all experiments. Mice were maintained in a temperature-controlled environment ($25 \pm 1^\circ\text{C}$) on a 12 hr light/dark cycle and free access to foods and water. Neomycin sulfate dissolved in a saline was administered s.c. at a dose of $50 \mu\text{mol}$ (approx. 31 mg)/kg/day at 12:00 to 1:00 p.m. once daily for 10 or 15 days. Control groups were injected with saline alone. Mice were 6 to 8 weeks old (27–34 g) at the start of saline or neomycin injection. All experiments were carried out 21–28 hr after last saline or neomycin injection except for rota-rod test and traction test.

Rota-rod test: According to the method of Dunhum and Miya (1957), we carried out the rota-rod test by means of a rota-rod apparatus (Shinano Seisakusho, SN-497, Tokyo, Japan) just before each treatment with saline or neomycin. The diameter of the rod was 30 mm and the speed of the rotation was 8 rpm. We measured the elapsed time (sec) from putting the mouse on the rod until falling off the rod. Such measurement was repeated three times and the mean value was calculated. Animals of 60 sec or more elapsed time were distinguished into a positive response individual and those of less than 60 sec into a negative response individual. The percentile value was calculated from the following formula;

$$S = N_s / N_t \times 100$$

where N_s is the number of positive response individuals and N_t is that of all individuals tested.

Traction test: The Julou-Courvoisier traction test was carried out as described by Osbahr, III *et al.* (1979). Mice were placed so as to grasp with

their forepaws at the taut wire (2.5 mm diameter and 25 cm length) strung 20 cm high horizontally. If mice grasp the wire within 10 sec after the placement and hang, the muscular function was judged as no effect and if mice do not grasp the wire and drop from the wire, the muscular relaxation was judged as positive.

Tension recordings: Referring to the method of Bulbring (1946), tension recordings were performed on the isolated left phrenic nerve-hemidiaphragm preparations from mice treated with saline or neomycin for 10 or 15 days. The preparation was soaked in Krebs-Ringer solution of the following composition (mM): NaCl 136, KCl 5, CaCl_2 2, MgCl_2 1, NaHCO_3 15 and glucose 11. The solution was bubbled with a mixture of 95% O_2 and 5% CO_2 and kept at pH 7.3 and 36°C . One of the site of insertion into the rib cage of the preparations with its phrenic nerve was fixed and the other central tendon was attached to a force displacement transducer (Nihon Kohden, TB-652T, Tokyo, Japan). The phrenic nerve was stimulated through a suction electrode connected to an electronic square pulse generator (Nihon Kohden, SEN-3201) with a constant voltage output. All stimuli were supra-maximal threshold of 0.1 msec duration at 0.1 Hz. The twitch tensions of diaphragm were recorded on a thermal recorder (Nihon Kohden, RJG-4122).

Statistical analysis: Statistical significant difference between two means was determined by Student's *t*-test except for a comparison between two regression parameters.

RESULTS

The repeated treatments with neomycin caused no death and no significant effect on the body weight in mice (data not shown).

Rota-rod test was carried out daily to detect the neurological deficit in small experimental animals. The results in Fig. 1 showed the principle effect with the time-dependent manner in both cases of control and neomycin treatment. After injection of 10 times, *S* reached at plateau level in both cases. By the regression analysis, there was good correlation between the injected times and *S* in both groups. However, the slope and the intercept of the regression curve were not

Fig. 1. Effect of repeated treatments with saline or neomycin on the motor coordination assessed by rota-rod test. Control (○—○): $n=13$, $r=0.984$. Neomycin (●—●): $n=39$, $r=0.981$. Ordinate scale: Percentile value obtained from the following formula; $S=Ns/Nt \times 100$ (see in detail Materials and Methods). Abscissa scale: Injected times (day) with saline or neomycin.

affected significantly by repeated treatments with neomycin.

As shown in Table 1, repeated treatments with neomycin did not induce the muscle relaxation assessed by traction test.

As shown in Fig. 2, twitch responses to stimulations of phrenic nerve occurred on control and neomycin-repeated treatment preparations. In a standard bathing solution without any drugs, basal twitch tensions (g) were 1.9 ± 0.2 ($n=21$), 2.0 ± 0.2 ($n=5$), 1.8 ± 0.2 ($n=24$), 2.0 ± 0.4 ($n=17$) in control, neomycin-1, neomycin-10 days and neomycin-15 days treatment preparations, respectively. There were no significant differences among them, suggesting that repeated treatments with neomycin did not affect the basal twitch tension in response to nerve stimulation. In cumulative additions of *d*-tubocurarine to a standard bathing solution at intervals of 5 min, the twitch responses evoked by phrenic nerve sti-

Table 1. Effect of repeated treatments with saline or neomycin on the muscular function assessed by traction test.

Treatments	days	No. of mice	
		without effect	with relaxation
Saline	10	10	0
	15	9	1
Neomycin	10	10	1
	15	10	1

mulations were inhibited with increasing concentrations of *d*-tubocurarine in both cases of control and neomycin treatment preparations.

As shown in Fig. 3, the final concentrations of *d*-tubocurarine producing complete twitch inhibition were different in four treatment cases. Then, we estimated the relationship between

Fig. 2. Typical data of the effect of addition of *d*-tubocurarine (*d*-Tc) to a standard bathing solution on the twitch tension of diaphragm evoked by phrenic nerve stimulation in the cases of repeated treatments with saline (Control, upper trace) or with neomycin for 15 days (NMC-15, lower trace). Closed circles show the addition of *d*-Tc at the concentration of bottom numerals (μ M). Arrows show the replacement to a fresh standard bathing solution.

Fig. 3. Effect of repeated treatments with neomycin on the relationship between the twitch inhibition and the concentration of *d*-tubocurarine (*d*-Tc). Control (○): Saline treatments ($n=6$), NMC-1 (■): neomycin treatment for 1 day ($n=5$), NMC-10 (▲): neomycin treatments for 10 days ($n=7$) and NMC-15 (●): neomycin treatments for 15 days ($n=8$). Ordinate scale: percentile twitch tension after an addition of *d*-Tc to a bathing solution. Abscissa scale: natural logarithmic values of the concentration of *d*-Tc (μ M). Each point shows the mean and S.E.. Each asterisk shows the significant difference from control at $P<0.05$.

twitch inhibition and concentration of *d*-tubocurarine. This relationship was obtained as sigmoid in all cases. On the preparation treated with neomycin one time, there are no significant differences in control. Repeated treatments with neomycin shifted the concentration-response curve to the lower *d*-tubocurarine dependently on the term of neomycin treatment. IC_{50} (μM) of *d*-tubocurarine was 1.506, 1.391 and 1.167 in control, neomycin-10 day and neomycin-15 day treatments, respectively.

DISCUSSIONS

Even if the neuromuscular junction was damaged or disturbed by drugs, there is a case that the indirectly evoked twitches show normal size height, because a large safety margin exists in the neuromuscular junction (Paton and Waud, 1967). This safety margin may be revealed by the treatment with *d*-tubocurarine. If the safety margin becomes smaller than normal level by the treatment with neomycin, the concentration of *d*-tubocurarine to produce the complete twitch inhibition may be lower in neomycin treatment cases. At this experiment, we obtained the results that the motor coordination assessed by rota-rod test, the muscle relaxation by traction test and the basal twitch tensions evoked by nerve stimulations were not affected by repeated treatments with neomycin. These results may reflect a safety margin at neuromuscular junction. However, the concentration-response curve of *d*-tubocurarine was shifted to the lower concentration, dependently on the times of neomycin injections. These results suggest that repeated treatments with neomycin reduce the safety margin at neuromuscular junction. Furthermore, the results obtained in this study well resembles with those reported by Durant *et al.* (1981). Their results were summarized as follows; under the neuromuscular block of 80–90% after a preadministration of neomycin (25–30 mg/kg) to cats, the ED_{50} of *d*-tubocurarine significantly decreased the indirect twitch tension of the tibialis anterior muscle. Our experiment was carried out about 24 hr after the last neomycin injection and found that the residual effect of neomycin still remained even at 24 hr after 10 or 15 daily injections with neomycin.

We could consider two possibilities from the present results that repeated treatments with neomycin enhance the inhibitory effect of *d*-tubocurarine; one is reduction of the release of ACh from the motor nerve terminals, and the other is decrease in the number of ACh receptors and/or change in the nature of ACh receptors at the end-plate. However, in the present study, we could not clarify the mechanism of this residual enhancement of the inhibitory effect of *d*-tubocurarine by repeated treatments with neomycin.

In summary, repeated treatments with neomycin *in vivo* enhances the sensitivity to *d*-tubocurarine at the neuromuscular transmission without eliciting motor incoordination, muscle relaxation and basal twitch change, i.e. reduction of the safety margin at neuromuscular transmission.

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