Neuromuscular block after intra-arterially injected acetylcholine

2. Effects of ACTH treatments as possible detectors of desensitization level in the receptor site

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SUMMARY It has been suggested that the effect of ACTH in myasthenia gravis may be ascribed to an action involving neuromuscular transmission which favours repolarization processes, with a tendency towards hyperpolarization of the membranes of muscle fibres and motor nerve endings. A similar mechanism has been postulated for the action of ACTH in epilepsy (Klein, 1970). A direct or indirect action on nerve membrane would interfere with depolarization. There is evidence of raised concentration of intracellular potassium and increased outflow of sodium ions which would cause hyperpolarization of the membrane. This paper studies the effect of ACTH on the late block of neuromuscular transmission caused by acetylcholine (ACTH).

Intra-arterial injection of acetylcholine brings about changes in the amplitude of muscle action potentials in response to repetitive motor nerve stimulation due to depolarizing and desensitizing blocks (Grob, Johns, and Harvey, 1956; Grob and Johns, 1961; Pinelli, Tonali, Gambi, and Pelliccioli, 1970; Gambi and Tonali, 1971).

The variable range of parameters suited for the determination of the magnitude of such

blocks was identified, and investigations were made on the effect of ACTH treatment of myasthenia gravis.

METHODS

Seven patients for whom ACTH treatment was an adequate therapy were selected in the group of 13 patients who were given ACh injections with the

FIG. 1. Pattern of action potentials of the opponens pollicis muscle in response to intermittent repetitive supra-maximal stimulation of the median nerve after intra-arterial injection of 10 mg ACh in a subject having no disturbances of neuromuscular transmission after ACTH treatment (700 i.u.) (the subject is the same as shown in Fig. 1 of Tonali and Gambi, 1973). Stimuli of 0.1 msec were delivered in trains of four, 50 msec apart. Intervals between successive trains varied from 1 to 15 seconds. From left to right, responses to a train of stimuli are recorded at the beginning, at the climax of prompt depression, of recovery and of late depression, and after one hour's interval.

FIG. 2. Time course on a logarithmic scale of amplitude of muscle action potentials in response to intermittent repetitive supramaximal stimulation of the median nerve after intra-arterial injection of 10 mg ACh. The diagram shows the mean values (related to 10 mV) recorded in the subjects examined after ACTH treatment for the first (●—●) and fourth (○—○) potential.

results reported in the previous paper (Tonali and Gambi, 1973).

ACTH was injected intramuscularly at a daily dosage of 100 i.u. for a continuous period ranging from five days to a week; a single subject was given an overall dosage of 450 i.u. The ACh test was always performed at a 10 mg dose, not more than 12 hours after the last injection of ACTH.

The technique, procedure, and evaluation of results were the same as in the previous paper (Tonali and Gambi, 1973). In no subject did ACTH treatment evoke any alteration in the electrolytic pattern and the other biological parameters detectable by laboratory analyses. Data concerning ACh injection after ACTH treatment will be set forth following the same scheme as in the first part of our study.

RESULTS

'PROMPT' DEPRESSANT EFFECT OF ACh (Figs 1 and 2). 1. Reduction in amplitude of evoked potentials began in 7 ± 0.6 seconds for the first and 6.7 ± 0.59 seconds for the last in a train of four nerve stimuli after intra-arterial injection of ACh. Such a reduction attained its peak ($-78.3 \pm 6.51\%$ for the first potential and $-79.6 \pm 6.23\%$ for the fourth potential) after a lapse of 10.6 ± 1.08 seconds in both instances.

2. After maximum downfall a prompt recovery took place, and potentials were restored at levels not lower than recorded immediately before the beginning of downfall, after an average interval of 28.6 ± 3.92 seconds and

26.7 ± 4.73 seconds for the first and the fourth potential respectively.

As a follow-up, a further increase in amplitude was registered and percentage mean maximal values were reached—namely, $+1.1 \pm 16.31\%$ and $+0.57 \pm 18.79\%$ for the first and for the fourth potential respectively—after an interval in the range of 46.3 ± 7.68 seconds and of 31.3 ± 5.76 seconds respectively for the first and for the fourth potential.

The comparison between records taken before and after ACTH treatment did not show any substantial difference, even when the variations detected in subjects submitted to a second test are taken into account. It is worth pointing out, however, that after ACTH treatment prompt depression set in and reached its peak slightly earlier and had a greater amplitude ($+5\%$), whereas recovery took up a longer interval only for the first potential.

LATE DEPRESSANT EFFECT OF ACh 1. *Onset of late depression* After recovery from prompt depression there was a second reduction in amplitude of muscle potentials. This could occur immediately or after a period in which potentials remained at the same value. This stage began in the range of 229.3 ± 71.22 seconds for the first potential and of 173.5 ± 51.09 seconds for the fourth potential respectively.

2. *Amplitude of late depression* This amounted to $-7.22 \pm 2.01\%$ for the first potential and to $-6.66 \pm 3.31\%$ for the fourth potential, and it was reached after an interval of 989.3 ± 326.06 seconds and of 925.7 ± 148.27 seconds respectively.

3. *Duration of late depression* Late depression lasted for $1,588.1 \pm 470.48$ seconds for the first potential and 840.2 ± 397.33 seconds for the fourth potential.

4. *Recovery from late depression* After 3,600 seconds potentials attained mean percentage values in the order of $-1.11 \pm 4.63\%$ and of $-1.85 \pm 4.26\%$ respectively for the first and for the fourth potential.

Late depression accordingly showed quite noticeable variations in the subjects who received ACTH treatment. Compared with the tests per-

formed before treatment and taking into account individual variations described in the first part of this study, the beginning of depression is delayed three to six times as much and its amplitude is 50 to 75% reduced. It is significant that late depression also comes to its peak after an interval at least 250 seconds—namely, 27%—longer than in the tests made before treatment.

As far as the duration of depression is concerned, uneven variations are observed in the first and in the fourth potentials of the train, and are practically nil in the evaluation of other factors. Only in the first potential is it possible to notice a tendency towards increasing duration. Finally, recovery from late depression seems to be more conspicuous, since the amplitude of evoked potentials is restored on mean percentage levels not lower than 2% with respect to the initial amplitude.

No relationship was observed between ACTH dosage and the modifications in late depression previously mentioned.

CONCLUSIONS

The data gathered in our investigation on the effects of large doses of intra-arterially injected acetylcholine (ACh) demonstrate that ACTH treatment does not substantially affect prompt depression, and that highly significant alterations are brought about in late depression. Such evaluations are based on a statistical analysis and plotting of our records and data, taking into account not only records taken before treatment, but also the differences detected in subjects who received two injections of ACh. Modifications in late depression are basically identified by a delay in the beginning of depression and by a reduction in amplitude, which in two instances disappears altogether.

The finding that ACTH affects 'late' depression only suggests that different mechanisms underlie the two stages of development of ACh-elicited block. Hence, indirect support is given to the hypothesis formulated by Elmquist, Hofmann, Kugelberg, and Quastel (1964), Gissen and Nastuk (1966), and by Thesleff (1966) that late depression is not controlled by merely depolarizing mechanisms but rather is a consequence of 'desensitization' of the receptor site in the end-plates. According to this hypothesis, ACTH

would reduce such desensitization processes and receptors would more rapidly resume their ACh-sensitive behaviour. It would thus appear that the ACTH effect may be considered as a test for the evaluation of the desensitization of the receptors. Needless to say, such indication may be extremely important in disturbances of neuromuscular transmission, with particular reference to some forms and/or stages of myasthenia gravis which are characterized by poor response to anticholinesterase medication.

In this connection we may point out that in a case previously described by the present authors (Pinelli, 1970; Gambi and Tonali, 1971), an ophthalmoplegic syndrome of a myopathic character complicated by myasthenia gravis insensitive to anticholinesterase compounds and to ACTH, the latter caused a marked enhancement of 'late' depression. This would suggest that in this specific case ACTH did not result in a reduction of desensitization processes: on the contrary, the latter would appear to be intensified, possibly because of a greater lability of receptors related to a probable myopathic disturbance.

It is quite apparent that only systematic and thorough investigations on myasthenic patients will prove whether or not Grob's test may detect the state of 'desensitization' of receptors for which anticholinesterase and ACTH treatment would be of no avail.

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