Neuromuscular block after intra-arterially injected acetylcholine

1. Introduction, methods, and technique

P. TONALI AND D. GAMBI

From the Clinic of Nervous and Mental Diseases, Catholic University, Rome, Italy

SUMMARY The neuromuscular depolarizing block induced by intra-arterially injected ACh was studied to determine the variability in the same subject and in different subjects without disorders at the motor end-plate. Amplitude of action potentials of the opponens pollicis muscle evoked by intermittent repetitive supramaximal stimulation of the median nerve at the wrist were recorded for one hour from the beginning of ACh injection. The features of prompt and late depression stages after the injection were analysed statistically. Re-testing of the same subjects after a while shows that, in spite of all efforts to maintain the same experimental conditions, variations do occur in late depression. Time course and duration are particularly affected, while the degree of depression is altered but slightly. The presence of such variations limits this test to evaluation of the influence of other factors only within their already established statistical limits.

The intra-arterial injection of large doses of acetylcholine (ACh) in normal subjects produces a block of neuromuscular transmission due to prolonged depolarization of the motor end-plates. Previous investigations (Grob, Johns, and Harvey, 1956; Grob and Johns, 1961) showed that for doses of ACh exceeding 1 mg such 'depolarization' block showed constant features detected by measuring the amplitude of muscle action potentials in response to motor nerve repetitive stimulations. In effect, a prompt and short-lived depression of response starts a few seconds after intra-arterial injection of ACh and is followed by a temporary recovery, after which the so-called 'late depression' takes place, which has a more prolonged effect.

The exact characteristics of these phenomena and their reproducibility in the same patient have not been the subject of systematic and thorough investigations as yet, and there has been practically no follow-up of the contributions made by Grob and his associates. It is apparent, therefore, that the analysis and reproducibility of the data under consideration are essential for the evaluation of their possible modifications.

METHODS

The effect on neuromuscular transmission of ACh doses exceeding 1 mg injected into the brachial artery was studied on the basis of the amplitude of the action potential of the opponens pollicis muscle in response to intermittent repetitive supramaximal stimulation of the median nerve at the wrist according to the technique suggested by Grob and his associates.

ACh injection was administered to 13 patients, of whom three were women, with an age range of 22 to 69 years. Eight subjects were affected by disseminated multiple sclerosis, two by polyneuritis, one by cerebral neoplasm, one by compression of the spinal cord, and one by rheumatic polymyalgia. None of them showed any clinical or electromyographic signs of a lesion of the median nerve or of the muscles it innervates.

In all patients ACh was injected intra-arterially: in five subjects the injection was repeated at two days' interval at least. All tests were performed in basal conditions, at a constant ambient temperature, and care was taken to avoid any significant variations in arterial pressure.

Response action potential was picked up by means of surface electrodes made of small silver plates,

4 mm in diameter. The recording electrode was strapped over the belly of the muscle and the indifferent electrode was strapped to the base of the first phalanx of the thumb. The electromyograph used was a rack mounted 4 Channel SDC 3R model Medelec. The optimal position of the stimulating cathode was the one which enabled the widest simple-shaped potential to be evoked in response to supramaximal stimulation. The thumb was fixed in slight abduction by means of a special fixing support placed on the back of the hand.

The median nerve was stimulated at the wrist by a bipolar electrode using rectangular electrical pulses of 0.1–0.2 msec duration at supramaximal intensity—that is, at a level 50% greater than maximal intensity.

Stimuli were delivered in trains of four, 50 msec apart: the various trains were spaced at intervals of one to 30 seconds, and the whole test was accomplished within one hour's time. The stimulating electrodes were kept *in situ* by hand, since abundant sweating was induced by ACh injection and frequent cleaning of the skin with ether was needed. A sphygmomanometer cuff was placed around the upper arm at a pressure of 300 mm Hg, so as to occlude blood flow, and ACh was injected into the brachial artery at the elbow, as rapidly as possible. Pressure in the cuff was immediately released and set slightly above venous pressure with a view to retarding the escape of the drug into the systemic circulation. After a latent period of two to three seconds the injection was followed by an intense vasomotor reaction involving the forearm and the hand, with sweating, marked flushing, and a painful sense of burning. At the same time involuntary movements of the fingers and the hand were observed, especially flexion twitches, and were followed by a paralysis which tended to regress 10 seconds later. Regression

of vasomotor phenomena was somewhat slower and took five to 10 minutes. Repetitive stimulation was started at the time of the injection and continued at the frequency of one train per second in the first minute. Afterwards the time interval was extended to 5, 10, 15, and 30 seconds up to the 60th minute. When evaluating the amplitude of muscle response, we took account of the initial negative deflection of the first and fourth potential of each train throughout the test.

RESULTS

CONTROL OF PATTERN OF MUSCLE ACTION POTENTIALS IN RESPONSE TO TRAIN OF REPETITIVE STIMULATION The amplitude of muscle action potential responses to supramaximal repetitive stimulation based on the above technique sometimes shows variability in nature and degree, such as to compromise the correct interpretation of data. For this reason, we made control studies on subjects without abnormalities in the region of the neuromuscular end-plate.

To this effect, four subjects were examined in whom stimulation was applied, without the previous injection of ACh, in trains of four stimuli at 20 per second delivered every second for periods of two to five minutes. For the purpose of our investigation, all evaluations were based on the first and fourth potentials of each train, which may provide the most reliable comparative indication of any disturbances involving neuromuscular transmission.

Our findings were (1) The first potential of the train exhibited variations in amplitude on both sides ranging from +1.2% to +3.5% and from

FIG. 1. Pattern of action potentials of the opponens pollicis muscle in response to intermittent repetitive supramaximal stimulation of the median nerve after intra-arterial injection of 10 mg ACh in a subject having no disturbances of neuromuscular transmission. Stimuli of 0.1 msec were delivered in trains of four, 50 msec apart. Intervals between successive trains varied from 1 to 15 sec. 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 our subjects for the first (●—●) and fourth (○---○) potential.

−2.5% to −5.09% respectively, the mean values being $+1.52\%$ ($SD \pm 1.25$) and -3.72% ($SD \pm 1.33$). (2) The fourth potential's values were in the range of $+2.08\%$ to $+2.6\%$ and of -2.6% to -8.3% , with a mean of $+1.17\%$ ($SD \pm 1.18$) and -4.40% ($SD \pm 2.30$) respectively.

EFFECTS OF INTRA-ARTERIALLY INJECTED ACh
Doses of 2, 5, and 10 mg were adminis-

tered. Since the 2 mg and 5 mg injections each failed to induce constant and evident effects in the same patient and in different subjects, the 10 mg dosage was selected as the only one capable of eliciting a constant response, even if quantitative variations in different subjects could still occur.

The pattern of response in prompt and late depression will now be described (Figs 1, 2).

Muscle action potentials before prompt depression In the 13 subjects who were given the first intra-arterial injection of 10 mg ACh, the variability in the amplitude of first potential evoked by a train of stimuli fell within the range of +1.6% to +7.01% and of -0.6% to -7.5%, with mean values of $+1.21 \pm 1.97\%$ and of $-3.08 \pm 2.41\%$ respectively. For the fourth potential the variable range was +1.2% to +8.1% and -0.4% to -7.4%, with mean values of $+1.77 \pm 2.54\%$ and of $-2.80 \pm 2.39\%$ respectively.

Five subjects out of 13 received a second ACh injection; the amplitude of the first potential varied from zero to +2.1% and from -2.1% to -5%, the mean being $+0.42 \pm 0.84\%$ and $-3.12 \pm 1.83\%$ respectively. For the fourth potential variability was in the range of +2.1% to +9.2% and of -2.1% to -9.6%, with mean values of $+3.20 \pm 3.13\%$ and of $-3.08 \pm 3.54\%$ respectively.

Comparison of control data with data detected in the group of subjects with the second injection of ACh shows inhomogeneous findings, since only in the fourth potential did a decreased tendency to negative deflection appear in ACh tests. However, variations in the two types of deflection in the three tests were always within the range of 3%.

Prompt depressant effect of ACh Reduction in the amplitude of induced muscle action potentials set in after a mean interval of 7.3 ± 0.48 seconds after intra-arterial injection of ACh. Such interval was the same for the first and fourth potential and has been calculated taking into account the moment in which potential amplitude still had a higher percentage value than the maximal negative deflection of potential amplitude recorded in basal conditions.

Fall in amplitude of the first and the fourth potentials was maximal after a mean interval of 11.3 ± 0.53 seconds: based on the initial response (M), deflections of $-73.5 \pm 4.85\%$ and of $-73.2 \pm 5.19\%$ were recorded for the first and the fourth potentials respectively.

In the five subjects mentioned in the previous section, prompt depression after the second injection of ACh started after an interval of 8 ± 1.54 seconds both in the first and the fourth potential. A maximal reduction of $-72.3 \pm 9.87\%$

(first potential) and of $-73.96 \pm 9\%$ (fourth potential) was recorded after a mean period of 13 ± 3.46 seconds. The first and the fourth potentials therefore exhibited very much the same pattern in the same group of subjects, and the comparison between the two tests only points out a difference in the variability range of maximal reduction in action potential amplitude equalling 5%.

After maximal fall in amplitude a rapid recovery took place and potentials were restored to the same values as recorded immediately before the onset of depression: this occurred after a mean period of 29.9 ± 4.18 seconds for the first potential and of 28.6 ± 3.45 seconds for the fourth potential. A further increase was also observed until a climax was reached: the mean of top values (expressed as percentage of the initial potential) was $-2.9 \pm 8.61\%$ and $-0.7 \pm 9.81\%$ respectively for the first and the fourth potentials, and was attained after a mean interval of 31.8 ± 4.16 seconds and of 33.2 ± 4.10 seconds respectively.

In the five subjects who were given a second injection of ACh, recovery from prompt depression took place 32 ± 15.23 seconds later (first potential) and 25.80 ± 6.58 seconds later (fourth potential). Recovery reached a peak after an interval of 37.20 ± 11.83 seconds and 44 ± 28.42 seconds for the first and the fourth potentials respectively.

Mean amplitude at this time showed an increase of $+1.04 \pm 2.45\%$ and of $+3.66 \pm 2.99\%$ respectively over the initial amplitude of the first and the fourth potentials.

Accordingly, the analysis of data obtained in each group of patients suggests that, in this stage of the phenomenon, uneven variations in the several parameters may occur, not only in the two subsequent tests, but also within the same test.

Late depressant effect of ACh Onset of late depression Amplitude of action potential evoked in muscle reaches a maximum in the recovery from prompt depression, then falls again: this drop sometimes occurs immediately, sometimes after an interval in which amplitude is maintained at a stable level. The onset of this new stage is featured by a fall of potential amplitude below its maximal value. The time interval

calculated on 13 subjects from the beginning of the injection had a mean of 40.3 ± 5.37 seconds and of 39.5 ± 4.3 seconds respectively for the first and the fourth potentials.

In the five subjects who were given a second injection of ACh, the time interval was in the range of 55 ± 22.69 seconds and of 59 ± 31.58 seconds for the first and the fourth potentials respectively.

Along with a substantial agreement between the values of the first and the fourth potentials in each group of patients, differences between the two series of tests were observed: based on mean values, they extend to 19.5 seconds (33% of the mean).

Features of late depression The characteristics and degree of potential reduction in this stage varied in each of the 13 subjects investigated. Potential amplitude dropped to a minimum ($-20.2 \pm 3.63\%$ for the first potential and $-21.6 \pm 4.24\%$ for the fourth potential) after an interval of 520 ± 315.65 seconds and of 675.7 ± 323.83 seconds respectively.

The five subjects selected for a second test exhibited minimum values of $-16.18 \pm 7.55\%$ and of $-15.56 \pm 7.86\%$, reached after an interval of 351 ± 249 seconds and of 646 ± 478.73 seconds, for the first and the fourth potentials respectively.

The greatest degree of late depression, plotted against the maximal amplitude of potential reached during the recovery from prompt depression, in the 13 subjects under consideration was $-15.27 \pm 6.28\%$ and $-19.14 \pm 6.13\%$ respectively for the first and the fourth potentials.

In the five subjects who received a control injection, these maximal values fell in the range of $-16.84 \pm 9.33\%$ (first potential) and of $-18.84 \pm 9.8\%$ (fourth potential).

This confirms that the patterns of the first and the fourth potentials are superimposable; moreover, the variations in the two tests had only a slight bearing on amplitude (not more than a 5% deviation), whereas alterations in time course were quite noticeable (169 seconds—that is, 33%—being the difference between the means).

Duration of late depression This is considered as the time interval from the onset of late depression to the beginning of recovery of potential amplitude from maximum fall, provided that

recovery be a progressive process without sudden deflections bringing it back to the same values as recorded at the beginning of recovery, or even lower. Duration of late depression was in the range of $1,169.4 \pm 520.15$ seconds and of 959.1 ± 405.57 seconds for the first and the fourth potentials respectively.

In the five subjects who had a control stimulation, duration of late depression was 505 ± 355.49 seconds and 587 ± 473.19 seconds for the first and the fourth potentials respectively.

When comparing the two tests, substantial differences emerge, which may even amount to 100%.

Recovery from late depression Based on amplitude of the evoked muscle potential recorded at the 3,600th second as against the initial potential, recovery amounted to $-8.3 \pm 2.82\%$ and to $-7.3 \pm 2.55\%$ for the first and the fourth potentials respectively. After the control injection given to five subjects, a mean of $-8.4 \pm 4.09\%$ and of $-5.7 \pm 6.32\%$ respectively was obtained for the first and the fourth potentials.

The results of the two tests may, therefore, readily be superimposed.

CONCLUSIONS

Our records and data seem to be fully consistent with the reports by Grob and associates concerning the evolution of depolarizing block due to intra-arterially injected ACh in the phases of prompt and late depression. It is significant, however, that in our experience comparatively constant effects could be obtained only with 10 mg doses of ACh.

The similar pattern of the first and the fourth potential is also confirmed, both exhibiting the same variations in amplitude, even though in our investigation this is less apparent in late depression. In the latter stage no gradual fall of potentials induced by trains of stimuli is observed. In agreement with the hypothesis of Grob and his associates, this suggests that late depression, like prompt depression, should not be attributed to a competitive block.

Re-testing of the same subjects after a while shows that, in spite of all our efforts to maintain the same experimental conditions, variations do occur in late depression. Time course and

duration are particularly affected, while the degree of depression is altered but slightly. The presence of such variations limits the application of this test to evaluation of the influence of other factors only within their already established statistical limits.

REFERENCES

- Grob, D., and Johns, R. J. (1961). Further studies on the mechanism of the defect in neuromuscular transmission in myasthenia gravis, with particular reference to the acetylcholine-insensitive block. In *Myasthenia Gravis. The Second International Symposium Proceedings*, pp. 127-149. Edited by H. R. Viets. Thomas: Springfield, Ill.
- Grob, D., Johns, R. J., and Harvey, A. M. (1956). Studies in neuromuscular function. *Johns Hopkins Hospital Bulletin*, **99**, 115-181.
- Pinelli, P., Tonali, P., Gambi, D., and Pelliccioli, G. (1970). Sul blocco neuromuscolare da introduzione endoarteriosa di acetilcolina. Effetti di trattamenti preliminari con ACTH. *Rivista di Neurologia*, **40**, 203-212.