V. TRANSMISSION AND BLOCK AT THE MOTOR ENDPLATE AND IN AUTONOMIC GANGLIA

THE INTERRUPTION OF NEUROMUSCULAR TRANSMISSION AND SOME OF ITS PROBLEMS

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A hundred years ago Pelouze and Claude Bernard (8) were very much impressed by the fact that although in an animal killed by a toxic dose of a variety of substances, the stimulation of the motor nerves still produced muscle contractions, yet in an animal killed by curare no such response occurred. Their own description is that: "sur l'animal encore chaud et mort depuis une minute les nerfs sont inertes comme sur un animal qui serait froid et mort depuis longtemps". I suppose we may say that this was the starting point of our knowledge of the neuromuscular junction. It is, however, only during the last 30 years that the theory of how a nerve impulse causes a propagated muscle impulse has been built up. Throughout this work the scientists responsible for it have found in curare an invaluable tool. On the other hand, the clarification of the process of neuromuscular transmission has proved equally revealing of the mode of action of curare, thus providing a beautiful example of reciprocal benefit to pharmacologists and physiologists.

We know, today, that curare interrupts neuromuscular transmission by competition with acetylcholine and I should like to summarize here the characteristics of such a block.

1. The block is not preceded by potentiation of the maximal twitch or by spontaneous fasciculations.
2. The muscle under the influence of *d*-tubocurarine cannot maintain a tetanus and,
3. shows an increased sensitivity to subsequent doses.
4. The block is readily antagonized by neostigmine and previous tetanization.
5. It is potentiated by any substance raising the endplate threshold to acetylcholine.
6. If curare is injected into the denervated tibialis of a cat no sign of contracture appears.

Until a few years ago the terms neuromuscular block and curarization were applied indiscriminately to the same process. Now, however, we know that competition with acetylcholine is not the only mechanism by which a substance can produce a neuromuscular block. Long-lasting acetylcholine-like effect at the motor end-plates will also block transmission. This was first realized during the study of the mode of action of decamethonium (1, 2, 6, 9). This substance if injected intravenously into an unanaesthetized cat will produce a flaccid paralysis very similar to that produced by *d*-tubocurarine. The neuromuscular block, however, when analyzed, is found to possess different characteristics.

1. The block is usually preceded by potentiation of the maximal twitch and by spontaneous fasciculations.

2. A tetanus, if present during the block, is well maintained and does not antagonize the block.

3. The block is not antagonized by neostigmine but

4. is readily antagonized by *d*-tubocurarine.

5. In the denervated muscle it causes a typical contracture.

6. The muscle shows increased sensitivity to at least the first two or three doses of decamethonium.

The analysis of the stimulant actions produced by decamethonium and of its actions on denervated mammalian muscle and on normal avian muscle has revealed that the substance mimics acetylcholine in its actions at the neuromuscular junction (2, 9). In the denervated tibialis of the cat the injection of decamethonium, like that of acetylcholine produces a double mechanical response consisting of a quick initial contraction followed by a prolonged contracture during which the directly stimulated twitches are depressed. The quick response is accompanied by an outburst of action potentials which is cut short with the onset of the slow contracture during which no more action potentials can be detected. The same response is obtained when acetylcholine or decamethonium is injected into an innervated gastrocnemius of a hen and if either substance is injected into the jugular vein of a chick it produces a typical response characterized by a retraction of the head and extension of the legs. In contrast to acetylcholine and decamethonium, *d*-tubocurarine produces the usual flaccid paralysis.

Burns and Paton (1), using the gracilis muscle in the cat, demonstrated by a skilful electrophysiological analysis that this likeness to acetylcholine rests in the ability of decamethonium to cause a persistent depolarization of the end-plate region. Using the same technique they demonstrated that all the principal features of block by decamethonium can be reproduced with acetylcholine in the presence of eserine. Again, as in the case of curare, the knowledge of the mechanism of neuromuscular transmission has helped in the analysis of the action of decamethonium which in its turn has thrown new light upon the action of acetylcholine.

Thus at this stage we had two clearly defined mechanisms of interruption of neuromuscular transmission, namely: (a) competition with acetylcholine and (b) long-lasting depolarization. What I should like to do now, is to give some account of the new facts which the use of depolarizing substances has brought to light.

First of all their study in various mammalian species has revealed the possibility of a third mode of neuromuscular block which combines the two processes just mentioned. The characteristic of this block is that the substance acts initially as a depolarizing substance, but during the blocking process its action changes into that of a substance competing with acetylcholine.

An analysis of the block produced in the monkey, dog and rabbit by decamethonium (10) shows that:—

1. A tetanus produced during the block is not well sustained and antagonizes the block.

2. The block is strongly antagonized by neostigmine and
3. deepened by d-tubocurarine.

These results might suggest that such a block is due to a curare-like effect. A more careful analysis shows, however, that the block is preceded by potentiation of the maximal twitch and by spontaneous fasciculations. At the same time if decamethonium is injected in a denervated muscle, the muscle will respond with a typical contracture. These are responses characteristic of a substance capable of depolarizing the motor end-plates. That this block does in fact pass through both depolarizing and competitive phases is further demonstrated by a new characteristic which is not seen with a substance acting solely by competition with acetylcholine *or* solely by depolarization; namely, a strikingly decreasing sensitivity of the muscles to second and subsequent doses. This picture is very similar to that observed in the cat when an injection of d-tubocurarine is interposed between doses of a depolarizing substance.

These results demonstrate that a single substance may produce different types of neuromuscular block in various mammalian species. It follows that differences must exist between the membranes of such muscle fibres in spite of the apparent similarity of their reaction to acetylcholine. But where these differences reside remains a matter of speculation.

In the cat, the soleus muscle is a red muscle and this redness is associated with slow contraction and easy fusion of tetanic stimuli. The tibialis anterior on the other hand, is a muscle in which white fibres predominate and this whiteness is associated with rapid contraction and fusion only at high frequencies of stimulation. These two muscles differ in their sensitivity to neuromuscular blocking substances. Whereas the tibialis is very sensitive to decamethonium, the soleus is particularly resistant, and whilst both muscles are sensitive to d-tubocurarine, the soleus is the more sensitive of the two (7). Decamethonium blocks the tibialis by long-lasting depolarization of the motor end-plates and it had been assumed that the soleus is blocked in a similar way. However, recent work done in collaboration with Dr. Peter A. Jewell (4) has shown that in the soleus decamethonium exhibits a dual mode of action having the characteristics just described. The substance starts its action by depolarizing the motor end plates but during the blocking process the action changes into that of competition with acetylcholine. These results demonstrate that differences between the neuromuscular junctions exist not only between the muscles of different species but even between various muscles of the same species.

In collaboration with Professor G. A. H. Buttle a study of the different muscles of the hen was made. Maximal twitches in response to indirect stimulation were simultaneously recorded from different muscles, for example, from the gastrocnemius muscle and from the pectoral muscle. These two muscles respond to an injection of decamethonium in a completely different way; the gastrocnemius muscle with a typical contracture, the pectoral muscle with a flaccid paralysis. Once again a differentiation between various muscles of the same species was made possible by the use of a depolarizing drug.

The mechanism of the contracture which occurs when acetylcholine or any depolarizing substance is injected into an innervated gastrocnemius of the hen

has never been satisfactorily explained. It is very difficult to understand why the same muscle should respond by a contraction, in other words by a propagated response to acetylcholine liberated by the nerve endings when the nerve is stimulated, and on the other hand by a contracture when acetylcholine is injected. There are at least two possible explanations:

(a) In such a muscle there is the same sensitivity to acetylcholine at the motor end-plate region as in the rest of the muscle membrane, or

(b) The sensitivity differs, but in any case the sensitivity of the rest of the muscle membrane is sufficient to allow a response after acetylcholine has been brought into contact with it.

Dr. W. L. M. Perry and I have tested these possibilities by measuring the potassium output of the muscle under the influence of a depolarizing substance, since depolarization of a membrane is associated with a decrease of its resistance and a consequent increase of permeability to cations. Skinned hind legs of normal and denervated cats and skinned legs of normal hens were perfused, the animals having first been loaded with $K^{42}Cl$. In the normal cat a dose of decamethonium sufficient to block completely neuromuscular transmission produced as a maximum an increase of 30 per cent in the rate of loss of potassium. We consider that this dose of decamethonium is quite sufficient to depolarize all the end-plates and part of the surrounding membrane of the muscles and that this rise of 30 per cent can be attributed to an increased exit of potassium across this zone. It follows therefore that any increased loss of potassium of more than 30 per cent must represent an increased flux across membrane other than that of the end-plate region. An injection of decamethonium sufficient to produce a contracture in the denervated mammalian muscle or in the innervated gastrocnemius of a hen produced an increase of 70 to 80 per cent in the rate of loss of potassium. Thus, results appear to justify our original hypothesis and possibly throw some light on the phenomenon of contracture in these muscles.

A further instance of the usefulness of depolarizing substances in analyzing events at the neuromuscular junction is supplied by the results obtained in myasthenic patients. Myasthenia gravis is a disease characterized by a gradually developing weakness of the muscles of the body. Different possibilities have been considered as the cause of this syndrome:

(a) Increase in cholinesterase activity,

(b) Diminished release of acetylcholine,

(c) Presence of a curare-like substance.

However, none of these theories has yet been substantiated by adequate proof. The use of depolarizing substances has once more proved a valuable source of information. Churchill-Davidson and Richardson (3) have obtained convincing evidence that the neuromuscular block produced by decamethonium in normal human beings is due to long-lasting depolarization of the motor end-plates but that the block produced by the same substance in myasthenics is the result of a dual mode of action. This means that, in the myasthenic as in the monkey, we are confronted with a transition from the picture of a depolarizing block to that of a competitive block. Consequently, a change has occurred in the muscle membrane

of the myasthenic patient. This explanation appears to fit the facts connected with the myasthenic syndrome as we know it.

Considering now the two processes of neuromuscular block (a) competition with acetylcholine, (b) depolarization, it may be said that a block produced by long-lasting depolarization has provided a great deal of information not accessible through the study of a block produced by competition with acetylcholine. However, while the interpretation of the results obtained with a competitive substance is relatively straightforward, since the response of skeletal muscles to such a substance is apparently uniform, the handling of a depolarizing substance, or of a substance having a depolarizing element, is much more difficult and the interpretation of the results obtained from different species and from different muscles needs great care.

Before concluding, one other point should perhaps be considered; i.e., whether the classical definition of a neuromuscular blocking substance can be applied to substances blocking by long-lasting depolarization. This definition requires that the substance should leave the nerve and muscle fibre unaffected. Information, however is accumulating which indicates that a muscle cannot be subjected to repeated depolarizations without suffering some harm (5). The nature of the harm done has not yet been determined. There is a practical conclusion to all this; namely that, both because of the non-uniform response of skeletal muscles to depolarizing agents and also because of their possible effect on the muscle fibre itself, these drugs must be applied with a very high degree of caution.

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