in nerve produced by neostigmine are primarily due to the action of preserved transmitter acetylcholine on motor nerve endings, whereas twitch augmentation and muscle repetition are primarily post-junctional events. The finding that these actions may be separated by acetylcholine antagonists indicates that slight differences exist between the cholinoceptors on the motor nerve endings and those on the post-junctional muscle endplate membrane. However, the difference is by no means as marked as that between ganglion and muscle receptors. The slight differences between the two populations of cholinoceptors at the pre- and post-junctional sides of the neuromuscular junction may be explained by the concept of "iso-receptors" as described by Jack (1970).

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The actions of depolarizing drugs and antagonists on developing muscle fibres *in vitro*

WILLIAM F. DRYDEN, SOLOMON D. ERULKAR AND GABRIEL DE LA HABA
Departments of Pharmacology, University of Strathclyde, Glasgow, U.K., and University of Pennsylvania, Philadelphia, U.S.A.

The stages in development of skeletal muscle fibre up to and after the point of innervation may be successfully studied using cultured cells. With this system, not only the morphological development may be studied, but also the pharmacological evolution of drug receptors, thus presenting an additional source of information into the details of drug action. The development of muscle fibres can be divided into three stages: the myoblast, a mononuclear cell containing no organized contractile elements; the myotube, formed by fusion of the myoblasts and consisting of a multinuclear tube, within the cytoplasm of which are appearing those formed elements characteristic of striated muscle. Contraction is usually shown midway through this stage. The final stage is the muscle fibre as is conventionally recognized. Contractures in response to acetylcholine, carbachol and decamethonium have been evinced from young myotubes (Dryden, 1970) and membrane depolarization resulting from Ach application to myotubes has also been shown (Fischbach, 1970). However, evidence of earlier receptor presence has not hitherto been reported.

Using conventional microelectrode techniques, the resting membrane potential of chick embryo myoblasts in primary culture was found to be $-8{\cdot}3$ mV (s.e. $\pm$ 0·2 mV). After fusion the resting potential rises over successive days in culture to a level of -46 mV (s.e. $\pm$ 2·8 mV). Application of acetylcholine to myotubes at all stages of development resulted in depolarization of the membrane. The minimum effective dose was 10^{-4}M but this decreased with development of the fibre. The response could be inhibited by prior addition of 100 μg/ml (+)-tubocurarine to the culture dish. A similar depolarizing response, also blockable by curare, was found with TMA, but not with bethanecol. The response of the mononuclear cells to the addition of acetylcholine was different. Instead of depolarization to 0 mV, a variable change in membrane potential was found. Dependent on the resting potential, either a depolarization or a hyperpolarization to an equilibrium value of -7 mV was observed. This response also could be blocked by the addition of (+)-tubocurarine.

It is postulated that the changes in myotube sensitivity reflect increasing permeability to ions which begin after fusion of the myoblasts. A nicotinic receptor, however, is present on the membrane of the myoblast before any other morphological or physiological development has occurred.

These results are in partial contrast to those of Harris, Heinemann & Tarakis (1971) who found that a cloned line of rat myoblasts had a resting potential of -70 mV and depolarized on application of Ach. The differences in findings are thought to be attributable either to species difference or, more likely, to partial maturation of the rat myoblasts after several passages in culture.

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