Chlorthalidone, administered intraperitoneally in single doses up to 200 mg/kg, exerted no significant effect ($P > 0.05$) on rat blood sugar at 1 or 2 h after injection when compared with an alkaline saline control. The low solubility of chlorthalidone necessitated the use of high concentrations of alkali (pH 12) to dissolve the drug in sufficient concentration to administer the large doses employed. A similar control solution was shown to produce a statistically significant ($P < 0.05$) hyperglycaemic response when injected intraperitoneally. Two hours after injection of chlorthalidone (200 mg/kg, i.p.) the intravenous glucose tolerance, as measured by the rate of disappearance of an intravenous glucose load (1 g/kg), was not significantly different from that of the controls ($P > 0.05$). In concentrations up to 200 μg/ml, chlorthalidone did not diminish glucose uptake by rat diaphragm muscle or epididymal adipose tissue incubated *in vitro*. Oral treatment with chlorthalidone (100 mg/kg day) for 28 days produced no deterioration of intravenous glucose tolerance when compared with pair-fed controls.

It is concluded that chlorthalidone is not hyperglycaemic in the rat in single, large doses when compared with a suitable alkaline control solution and does not influence the glucose tolerance of rats so treated, or treated orally for 28 days with large doses of the drug.

I wish to thank Dr. D. F. Ingham, Geigy Pharmaceuticals, for generous supplies of chlorthalidone, and Mr. John Boswell for excellent technical assistance.

REFERENCES

CARLINER, N. H., SCHELLING, J. L., RUSSELL, R. P., OKUN, R. & DAVIS, M. (1965). *J. Am. med. Ass.*, **191**, 535–540.
FOY, J. M. (1967). *Life Sci., Oxford*, **6**, 894–902.
REUTTER, F. & LABHARDT, A. (1961). *Helv. med. Acta.*, **28**, 487–495.
TABACHNICK, I. I. A., GULBENKIAN, A. & YANNELL, A. (1965). *Life Sci., Oxford*, **4**, 1931–1936.
WALES, J. K., GRANT, A. M. AND WOLFF, F. W. (1968). *J. Pharmac. exp. Ther.*, **159**, 229–235.

Mechanism of action of neostigmine at the neuromuscular junction

SANDRA N. WEBB
Department of Pharmacology, University of Strathclyde, Glasgow, C.1, U.K.

A study has been made, in cats under chloralose anaesthesia, of the relative abilities of a number of acetylcholine antagonists (hexamethonium, benzoquinonium, tubocurarine, gallamine and pancuronium) to inhibit the various facets of neostigmine's activity at the neuromuscular junction of the soleus muscle.

Neostigmine (150 μg/kg intravenously) increased the amplitude of the maximal twitch and gave rise to muscle fasciculations that were independent of the nerve stimulation. These effects were associated with repetitive action potentials both in the muscle and in the motor nerve, the latter being recorded antidromically in the soleus ventral rootlets (L7 and S_1).

Although, in large enough doses injected close-arterially into the muscle, all of the acetylcholine antagonists abolished all the effects of neostigmine, it was possible, by careful dosage with the different drugs, to dissociate muscle fasciculations and repetitive firing in the nerve from twitch augmentation and repetitive firing in the muscle. Hexamethonium depressed muscle fasciculations and repetitive firing in the nerve in doses slightly smaller than those necessary to diminish the augmented twitches and muscle repetition. On the other hand, gallamine and pancuronium depressed the augmented twitches and the muscle repetition in doses that allowed fasciculations and nerve repetition to continue. With both benzoquinonium and tubocurarine, it was not possible to demonstrate selectivity for any aspect of neostigmine's action, all effects being depressed simultaneously. The results indicate that muscle fasciculations and nerve repetitive firing are related events, as are twitch augmentation and muscle repetition. It is now known that acetylcholine can depolarize motor nerve endings as well as the post-junctional membrane of the muscle endplate (Hubbard, Schmidt & Yokota, 1965). Hexamethonium is relatively more active as a ganglion blocking drug than as a neuromuscular blocking drug, indicating its selectivity for neuronal receptors. Gallamine and pancuronium, however, have little ganglion blocking activity and are relatively selective for muscle receptors. Tubocurarine and benzoquinonium block both ganglionic and muscle receptors in comparable doses. It is concluded that muscle fasciculations and repetitive firing

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).

REFERENCES

HUBBARD, J. I., SCHMIDT, R. F. & YOKOTA, T. (1965). *J. Physiol.*, **181**, 810–829.
JACK, D. *Pharm. J.*, **205**, 237–240.

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.

REFERENCES

DRYDEN, W. F. (1970). *Experientia*, **26**, 984–986.
FISCHBACH, G. D. (1970). *Science*, **169**, 1331–1333.
HARRIS, A. J., HEINEMANN, S. & TARAKIS, H. (1971). *Nature, Lond.*, **231**, 296–301.