means of a constant voltage stimulator with supramaximal pulses of 100 μs duration at twelve/minute. Recordings were made on a smoked drum using a Starling spring-loaded heart lever. Two minute control recordings were made; the drug was added to the bath and the drum was stopped after a further 2 minutes. The bath was washed out 4 times during 30 minutes. Each type of experiment was repeated at least 3 times.

Prilocaine (20–80 μg/ml) and lignocaine (10–40 μg/ml) increased the twitch tension of diaphragms stimulated via their phrenic nerves.

This effect was not due to a direct action of the drug on skeletal muscle as neither drug increased the twitch tension of chronically denervated diaphragms stimulated supramaximally with pulses of 1 ms duration at twelve/minute.

It was noted that the increase in twitch tension produced in innervated diaphragms by prilocaine and lignocaine was rather less than that produced by neostigmine.

Acetylcholine was incubated with a 1 in 10 dilution of a haemolysate of human erythrocytes pretreated with prilocaine (1·5 mg) or lignocaine (1·0 mg), according to the method described by Burn (1952). When the mixture was added to a frog rectus preparation suspended in aerated Ringer solution at room temperature, a contraction resulted, whereas the same dose of acetylcholine and haemolysate failed to cause an effect. This suggested that the local anaesthetics had anticholinesterase activity, which has been confirmed biochemically using the method of Michel (1949).

Preincubation of the phrenic nerve-diaphragm preparations with mipafox (20 μg/ml) converted the increase in twitch tension produced by both drugs into a decrease, suggesting that at low concentrations, the effect of the drugs is probably the algebraic sum of their anticholinesterase and local anaesthetic actions.

We wish to thank Professor J. B. E. Baker for his encouragement and guidance, Mr. J. Frazer for technical assistance and Dr. R. F. Carlyle for a gift of mipafox.

REFERENCES

BURN, J. H. (1952). *Practical Pharmacology*. Oxford: Blackwell.

JINDAL, M. N. & PATEL, M. A. (1965). Neuromuscular action of some newly synthetized lignocaine analogues. *Indian J. Physiol. Pharmac.*, **9**, 57–62.

JONES, J. J. & LAITY, J. L. H. (1965). A note on an unusual effect of gallamine and tubocurarine. *Br. J. Pharmac. Chemother.*, **24**, 360–364.

KATZ, R. L. (1965). The pharmacological effects of prilocaine. *Acta anaesth. scand., supplement*, **16**, 29–35.

MICHEL, H. O. (1949). An electrometic method for the determination of red blood cell and plasma cholinesterase activity. *J. Lab. clin. Med.*, **34**, 1564–1568.

Theophylline and adenosine at the neuromuscular junction

B. L. GINSBORG and G. D. S. HIRST*
Department of Pharmacology, University of Edinburgh

Adenosine reduces the output of the transmitter from the terminals of the phrenic nerve (Ginsborg & Hirst, 1971). In an attempt to investigate the possible involvement of cyclic 3′–5′ AMP in this phenomenon the interaction of adenosine (0·025–0·25 mM) and theophylline (1·8 mM) has now been studied. Adenosine increases cyclic AMP concentrations in central nervous tissue and theophylline inhibits this increase (Sattin & Rall, 1970). In our experiments the mean quantal contents of endplate potentials recorded from fibres of the rat diaphragm have been measured, and we have found that there is indeed an interaction and that the effect of 0·05 mM adenosine is abolished by theophylline. The antagonism is not due to the increase in transmitter

output caused by theophylline (Goldberg & Singer, 1969) as such, since adenosine appears to reduce transmitter output by approximately the same extent whatever its original value.

G. D. S. H. is an I.C.I. Research Fellow.

REFERENCES

GINSBORG, B. L. & HIRST, G. D. S. (1971). Cyclic AMP, transmitter release and the effect of adenosine on neuromuscular transmission. *Nature, Lond.*, in the Press.

GOLDBERG, A. J. & SINGER, J. J. (1969). Evidence for a role of cyclic AMP in neuromuscular transmission. *Proc. natn. Acad. Sci. U.S.A.*, **64**, 134–141.

SATTIN, A. & RALL, T. W. (1970). The effect of adenosine and adenosine nucleotides on the cyclic adenosine 3′, 5′-phosphate content of guinea-pig cerebral cortex slices. *Mol. Pharmac.*, **6**, 13–23.

Accumulation of calcium at the motor endplate

R. H. EVANS (introduced by J. F. MITCHELL)
Department of Pharmacology, The Medical School, University of Bristol, Bristol BS8 1TD

Denervated rat diaphragm muscle shows an increase in uptake of ^{45}Ca in the presence of acetylcholine (ACh) which does not depend on membrane potential (Jenkinson & Nicholls, 1961). In addition, histochemical studies with the dye Alizarin red S seem to indicate that calcium accumulates at the motor endplate regions of mouse diaphragms treated with ACh (Lièvremont, Czajka & Tazieff-Depierre, 1968). This paper describes experiments which suggest that ACh and carbachol specifically influence the entry of calcium to the motor endplate region.

The influx of calcium at the motor endplate region of the mouse diaphragm, and its dependence on the presence of ACh or carbachol was examined, by means of ^{45}Ca. Mouse diaphragms which had been incubated at 37°C in Ringer solution (Lièvremont, *et al.*, 1968) which contained ^{45}Ca were dried in acetone and cleared in xylene, and the non-innervated and innervated zones of the muscle were separated. The radioactivity of ^{45}Ca in the muscle samples was determined by liquid scintillation counting, and the amount of calcium accumulated at the motor endplate region was calculated as nmol/diaphragm.

Probably because of hydrolysis, at least 0·5 mM ACh was required to produce a significant effect on the influx of calcium, but carbachol was effective in lower concentrations, and therefore it was used to demonstrate the time course of the accumulation of calcium at the motor endplate region. In the presence of carbachol, calcium accumulation increased progressively for 60 minutes. The initial rate of accumulation of calcium was $6{\cdot}20 \pm 0{\cdot}59$ (S.E. of mean) $\times 10^{-12}$ mol of calcium/motor endplate per hour (4998 ± 204 S.E. of mean, muscle fibres/hemidiaphragm). Calcium also accumulated at the endplates of diaphragms which had been denervated 18 days before incubation with ^{45}Ca Ringer solution and ACh.

To measure the efflux of accumulated calcium from the motor endplate region, diaphragms were pretreated for 30 min with 1 mM ACh in ^{45}Ca Ringer solution. ACh (1 mM) reduced the rate of efflux of the accumulated ^{45}Ca from the motor endplate region into tracer-free Ringer solution. The half time of washout for ^{45}Ca from diaphragms into calcium-free Ringer solution was 65 min for the calcium accumulated at the motor endplate region and 15 min for the calcium which had entered the non-innervated zone of the muscle.