(Evans, 1971). Some of the characteristics of this calcium accumulation at motor endplates of the mouse diaphragm have now been investigated.

Tubocurarine ($1{\cdot}2 \times 10^{-6}$M) produced 50% inhibition of the carbachol induced accumulation of labelled calcium at the junctional region of the diaphragm muscle. Suxamethonium (0·01mM) produced a similar effect to 0·1mM carbachol. Eserine sulphate (0·01mM) caused calcium to accumulate at the innervated zone of diaphragms in the presence of 0·01mM acetylcholine, a concentration which was ineffective in the absence of eserine. Eserine also caused the accumulation of calcium at the innervated zone of diaphragms stimulated via the phrenic nerve. Similar effects have been demonstrated by histological methods (Lièvremont, Czajka & Tazieff-Depierre, 1968) and Ahmad & Lewis (1961) have shown that decamethonium and suxamethonium increase the influx of labelled calcium into frog sartorius muscle.

Contractions recorded from mouse diaphragms stimulated via the phrenic nerve show that neuromuscular blockade occurs under similar experimental conditions to those which produce calcium accumulation. However, the accumulated calcium cannot be responsible for the blockade because transmission is restored long before the accumulated calcium can be washed out of the muscle. Measurement of the resting membrane potentials of mouse diaphragm fibres revealed the presence of miniature potentials at the innervated zone. When the muscle was perfused with carbachol (0·1mM) the miniature potentials disappeared and no change in resting membrane potential could be seen to occur. It therefore appears that the accumulation of calcium progresses at the innervated zone of the mouse diaphragm in the absence of motor endplate depolarization. Nevertheless, the presence of intracellular/extracellular ionic gradients must be necessary to produce the effect of carbachol on calcium accumulation because the effect was absent in muscles depolarized with high potassium Ringer solution and in muscles which had been cut transversely before incubation with carbachol.

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## Unchanged responses to local anaesthetics and quinidine in the denervated diaphragm

S. C. HARVEY* and H. P. RANG

*Department of Physiology and Biochemistry, University of Southampton*

The skeletal muscle membrane undergoes extensive changes after denervation. The changes in acetylcholine sensitivity and membrane resistance and capacitance are well known (Albuquerque & McIsaac, 1970). There also develops a partial resistance to tetrodotoxin (TTX) in mammalian muscle, such that the rate of rise of the action potential is decreased by about 50% in the presence of $10^{-6}$M TTX (Harris & Thesleff, 1971). To ascertain whether there is a similar change in sensitivity to other drugs that block the sodium channels, the effects of procaine, nupercaine, and quinidine were studied on the normal and denervated rat diaphragm.

Six to 24 days after section of the left phrenic nerve, the diaphragm was removed, and a strip was placed in a bath containing Liley's solution (Liley, 1956) at 30° C. Two microelectrodes were placed close together in the same fibre, one for recording and the other for hyperpolarizing and stimulating the fibre. The fibre was hyperpolarized to −95 mV for 30 sec and then stimulated by means of a rectangular depolarizing pulse. The action potential and its derivative were recorded. From 10 such recordings an average maximum rate of rise was calculated. The procedure was repeated one hour after adding the drug to the bathing solution, and the effect was expressed as the ratio of the maximum rate of rise after to that before the presence of the drug.

Log concentration-effect curves were nearly linear over a 10-fold range of concentration. For any one drug the line representing the denervated strips was not appreciably different from that representing innervated strips. EC50s were calculated; they are as follows: procaine, $4{\cdot}2\times10^{-4}$M innervated and $3{\cdot}6\times10^{-4}$M denervated; nupercaine, $3{\cdot}5\times10^{-6}$M innervated and $4{\cdot}0\times10^{-6}$M denervated; quinidine, $1{\cdot}9\times10^{-4}$M innervated and $1{\cdot}8\times10^{-4}$M denervated. In four experiments, the effect of procaine on denervated muscle was measured in the presence of $2{\cdot}5\times10^{-7}$ M TTX; the average maximum rate of rise in the TTX-resistant portion of the action potential was depressed to essentially the same relative degree as the normal action potential. The sensitivity to procaine thus seems to be the same in TTX-resistant and TTX-sensitive channels.

The results suggest that the change in the sodium channels following denervation is a selective one, resulting in a decrease in their sensitivity to TTX but not to the less specific local anaesthetics. It is possible that the changes are greater on the outer surface of the membrane, where TTX and acetylcholine are known to act, rather than the inner surface, where local anaesthetics are thought to act (e.g., see Frazier, Narahashi and Yamada, 1970).

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## Application of the dansyl procedure to study the metabolism and accumulation of 5-hydroxytryptamine in characterized neurones of *Helix pomatia*

N. N. OSBORNE (introduced by G. A. COTTRELL)

*Max-Planck- Institut für experimentelle Medizin, Arbeitsgruppe Neurochemie, Göttingen, Germany*

A microbiochemical method involving the use of dansyl chloride and microchromatography (Osborne, Briel & Neuhoff, 1971; Briel, Neuhoff & Maier, 1972; Osborne, 1973) was used to analyse the metabolism and accumulation of 5-hydroxytryptamine (5-HT) in characterized snail neurones (GSCs) which contain the amine (Cottrell & Osborne, 1970; Osborne & Neuhoff, 1973). $^{14}$C-Tryptophan ($10^{-7}$M radioactive tryptophan/ml over 2 h: 1 ml/snail) perfused through the central nervous system of the snail was taken up by the GSCs and also cells (buccal cells) which lack biogenic amines (Cottrell, 1970; Osborne, 1972). Only the GSCs however, have the capacity to metabolize $^{14}$C-tryptophan to form some 5-hydroxytryptophan and slightly more 5-HT. Electrical stimulation of the GSCs, strong enough to elicit cell firing, resulted in very much more 5-HT being produced, though there was a slight increase in the amount of labelled tryptophan and 5-hydroxytryptophan. Doubling the duration of stimulation and the amount of $^{14}$C-tryptophan perfused through the central nervous system had no great influence on the content of radioactive substances found in the GSC. Pretreatment of snails with p-chlorophenylalanine, an inhibitor of tryptophan-hydroxylase in the vertebrates (Koe & Weissman, 1966), though not interfering with the uptake of tryptophan into the GSCs, almost completely prevented the formation of 5-hydroxytryptophan. Perfusion of the central nervous system with $^{14}$C-5-HT ($10^{-7}$ mol radioactive 5-HT/ml: 1·5 ml/snail over a period of 4 h) showed that the GSCs accumulated amine, while the buccal cells lacked this ability. None of the $^{14}$C-5-HT within the GSCs were metabolized. In contrast, the whole central nervous system not only accumulated radioactive 5-HT, but also metabolized part of it to form 5-hydroxyindoleacetic acid.

The effects of imipramine, desipramine and nialamide upon the accumulation of $^{14}$C-5-HT into the GSCs were also studied, and the results are summarized in Table 1.