The depolarizing action of 5-hydroxytryptamine on sympathetic ganglion cells

D. I. WALLIS and B. WOODWARD*

Department of Physiology, University College, Cardiff

5-hydroxytryptamine (5-HT) is known to alter the excitability of sympathetic ganglion cells and can stimulate both excitatory and inhibitory neurones in the intestine (Gershon, 1967). In the work reported here, the action of 5-HT on the excised superior cervical ganglion of the rabbit has been examined by means of the sucrose-gap apparatus (Kosterlitz & Wallis, 1966). Ganglia were superfused with Krebs solution at 22° C and potential changes displayed on a potentiometric chart recorder.

The threshold concentration for depolarization was around 10^{-5} M 5-HT, which also depressed the height of the transmitted action potential. 10^{-4} M consistently produced depolarizations, but with this and higher concentrations repolarization began during the course of perfusion. This was followed by prolonged tachyphylaxis. Tachyphylaxis was largely avoided if injections of 5-HT were made into the perfusion stream. Estimates of dilution by the perfusion stream suggest an injection is dispersed in a volume of about 5 ml; thus, the standard injection of 80 μg gave a concentration around 40 μM. Responses to 5-HT were similar in magnitude and rate of onset to those elicited with acetylcholine and choline.

Depolarizations elicited by 40–80 μg 5-HT were followed by hyperpolarizations; the latter tended to decline after repeated exposures to 5-HT. Depolarization amplitude and area were related to the concentration, but response area tended to increase further even when amplitude had reached a maximum. The after-hyperpolarization was also concentration dependent; it was selectively depressed by ouabain.

Attempts to characterize the 5-HT receptors mediating depolarization are in progress with a variety of blocking agents. Picrotoxin and BOL 148, 10^{-5} to 10^{-4} M, is an effective blocking agent, as are morphine and phenyl biguanide in similar concentrations. Methysergide and LSD produced less complete block of the 5-HT responses at these concentrations. Atropine (3×10^{-6} to 3×10^{-5} M) reduced the amplitude and particularly the area of 5-HT responses, but hexamethonium (3×10^{-4} to 10^{-3} M) produced a considerable enhancement of both the amplitude and the area of the responses.

Leading from the proximal pole of the ganglion and a point on the cervical sympathetic nerve yields records of membrane potential change in the presynaptic terminals (Koketsu & Nishi, 1968). The presynaptic terminals were also depolarized by 5-HT, but these depolarizations were not usually enhanced by the presence of hexamethonium.

We conclude that ganglionic 5-HT receptors are located both pre- and postsynaptically and can mediate relatively large and rapid changes in membrane potential. The receptors of this preparation may provide a model for neuronal 5-HT receptors in general, including those of the CNS.

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Some characteristics of calcium accumulation at motor endplates of mouse diaphragm

R. H. EVANS

Department of Pharmacology, The Medical School, Bristol BS8 1TD

Carbachol causes the progressive accumulation of labelled calcium at the junctional region of mouse diaphragm muscle and this effect occurs in the absence of nerve terminals

(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.