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THE ACTION OF 5-HYDROXYTRYPTAMINE ON *MYTILUS* SMOOTH MUSCLE

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SUMMARY

1. In the nerve–muscle preparation, where catch was characteristically minimal, 5-hydroxytryptamine (5-HT) had no effect on resting membrane potential, junction potentials, spikes or contraction.

2. In muscle bundles, where catch was prominent, 5-HT did not change membrane potentials, but prolonged junction potentials and lowered the threshold for spike discharge and contraction.

3. In muscle bundles, exposed to high concentrations of 5-HT, depolarization evoked repetitive spikes, while in low 5-HT, spikes were seldom fired even with much greater depolarization.

4. In muscle bundles, the effective membrane resistance, $R_{eff.}$, decreased from 45–60 to 23–35 MΩ as 5-HT concentration was increased.

5. It is suggested that 5-HT may facilitate spike discharge by lowering the internal free Ca^{2+} concentration.

INTRODUCTION

Catch tension in the anterior byssus retractor muscle of *Mytilus edulis* L. can be relaxed by nerves which respond to electrical or pharmacological stimulation of the pedal ganglia (Van Nieuwenhoven, 1947; Takahashi, 1960). Catch can also be relaxed by applying brief, repetitive pulses directly to the muscle. Fletcher (1937) found a latent period, and a strength–duration curve for the relaxing effect of brief pulses, indicating that the relaxation is due to stimulation of intramuscular nerves. After the contractile response to acetylcholine and repetitive electrical stimuli was blocked by propantheline, Cambridge, Holgate & Sharp (1959) observed a purely relaxing response to direct electrical stimulation of the muscle, suggesting that the nerves mediating relaxation are non-cholinergic.

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When it was discovered that 5-HT reversibly relaxes catch in a range of concentrations between 10^{-9} and 10^{-6} M, and that *Mytilus* muscle contains 5-HT in a concentration of 1 μg/g tissue, it was suggested that release of this amine by nerve stimulation mediates relaxation of catch (Twarog, 1954). In *Mytilus* muscle a monoamine oxidase was detected which specifically metabolizes 5-HT (Blaschko & Hope, 1957). In 1960, Welsh & Moorhead identified 5-HT in pedal ganglia of *Mytilus* at a concentration of 15 μg/g tissue. 5-HT in low concentration acts similarly to nerve stimulation, is present in nerve and muscle tissue and is broken down by a specific enzyme within the muscle; thus 5-HT can be considered a possible mediator released by nerves which relax catch.

Evidence for the mediator function of 5-HT is incomplete. No pharmacological agent has been found which specifically inhibits relaxation by 5-HT and/or nerve stimulation. Lysergic acid diethylamide (LSD), which blocks 5-HT in many smooth muscles, relaxes catch in *Mytilus* (Hoyle & Lowy, 1956; Twarog, 1959). Lysergic acid butanol amide (UML) and brom-LSD, powerful 5-HT antagonists in other smooth muscles, do not, in *Mytilus*, in any concentration up to 10^{-3} M antagonize either applied 5-HT or relaxation via neural stimulation (B. M. Twarog unpublished). Northrop (1964) reported that high concentrations of UML decreased the relaxing effect of 5-HT. However, he noted that responses to electrical stimulation were not only 'more tonic' but 'weaker' in UML, suggesting that the block of 5-HT was not specific.

The action of 5-HT is to relax rather than inhibit excitation (Twarog, 1960*c*; Hoyle, 1964). 5-HT should probably be termed a 'relaxant' and the nerves which mediate relaxation of catch, 'relaxing nerves'. The terms 'inhibitor' and 'inhibitory nerves' are misleading because catch tension is observed after 'active state' has terminated (Jewell, 1959). Furthermore, in concentrations of 5-HT which relax catch, electrical and mechanical responses to stimulation are not diminished and frequently the single prolonged contraction is replaced by powerful rhythmic contractions (Twarog, 1954; Hoyle & Lowy, 1956). These rhythmic contractions apparently follow the discharge of spike potentials (Twarog, 1960*b*, 1967*b*).

Hoyle & Lowy (1956) have maintained an alternative view, namely that catch is a tetanic contraction of a special sort, and that 5-HT inhibits excitatory nerves. While they confirmed that 5-HT does not diminish phasic contraction, they proposed that tonic and phasic contractions are due to two independent excitor systems. They stated that 5-HT selectively inhibits the localized excitatory discharges of the tonic (catch) system, probably a ganglion plexus. However, the whole concept of the local nature of the potentials recorded by Hoyle & Lowy (1956), the relation between

these potentials and catch and phasic contraction, the existence of the ganglionic plexus reported by Bowden & Lowy (1955) and the inhibitory action of 5-HT have been questioned in detail (Twarog, 1960*a*, *b*, *c*, 1967*a*, *b*).

The action of 5-HT on the *Mytilus* muscle membrane is of great interest. Rhythmic electrical responses to stimulation in relaxing concentrations of 5-HT are characteristic. However, no changes in membrane potential level were recorded by the external 'oil gap' recording method used previously (Twarog, 1954, 1960*b*).

It has now been possible with micro-electrodes to examine further the effects of 5-HT on the muscle membrane. In the present study, membrane resting potentials, junction potentials and spikes were recorded and membrane resistance was measured as a function of 5-HT concentration.

METHODS

The methods of dissection, stimulating and recording from the nerve–muscle preparation and small muscle bundles have been described (Twarog, 1960*a*, 1967*b*). Membrane resistance was measured by passing current through the recording micropipette. Stimulus current was balanced by a bridge circuit derived from that designed by Araki & Otani (1955). In order to supply a constant current to the cell, the resistance of one bridge arm, in series with the micro-electrode, was 1000 MΩ. The range of applied current was between 10^{-10} and 5×10^{-9} A. The balance of the bridge was observed before and after the measurement, to check for changes in micro-electrode resistance. Unless the bridge remained in balance, the record was rejected.

RESULTS

Nerve–muscle preparation. Figure 1 shows the effect of 5-HT during stimulation of the nerve at 10 sec intervals, at low voltages. Resting

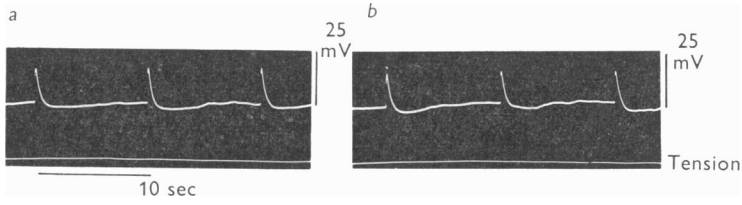

Fig. 1. The effect of 5-HT during repetitive neural stimulation. Upper trace, membrane potentials, lower trace, tension. Stimulus: 0·5 msec duration, 5 V, 1 per 10 seconds. (*a*) Control. (*b*) After 2 min in 5-HT 10^{-6} M.

and junction potentials and tension were continuously observed. No spikes were recorded and no contraction occurred. At 10^{-6} M, 5-HT did not affect the resting potential, junction potential or tension base line. Even after 15 min in 10^{-6} M 5-HT, no changes took place, although at this high concentration of 5-HT catch is totally abolished within 20 sec. In other ex-

periments, spikes and contraction were evoked by neural stimulation. No change was observed when the 5-HT concentration was increased to 10^{-6} M.

Small muscle bundles. In experiments with small muscle bundles, measurements were made at high 5-HT concentrations and compared with 'low' rather than zero concentrations of 5-HT. There were two reasons for this procedure. First, as described in a previous paper (Twarog, 1967*a*),

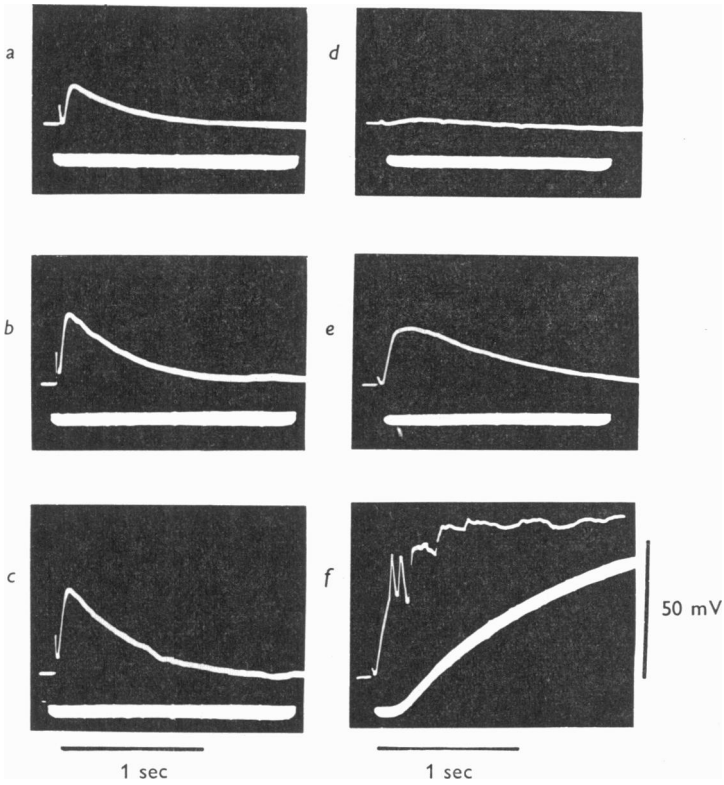

Fig. 2. The effect of 5-HT on responses to neural stimulation of fibres in a small bundle. Upper trace, membrane potential; lower trace, tension. Stimulating pulse in (*a*), (*b*), (*c*) 1 msec, increasing voltages to 22 V; in (*d*), (*e*), (*f*) 0·5 msec, increasing voltages to 12 V. (*a*), (*b*), (*c*) 10^{-8} M 5-HT; (*d*), (*e*), (*f*) 10^{-7} M 5-HT.

the level of catch can be precisely controlled if several factors, among these the 5-HT level, are held constant. In low 5-HT (10^{-9} M or 10^{-8} M), catch is prominent. In high 5-HT (above 10^{-7} M) catch is minimal. Second, the individual fibres of both the preparation with intact ganglia and the nerve–muscle preparation can readily be penetrated by a micro-electrode but the cells in the isolated bundles are extremely difficult to penetrate unless 5-HT is added to the perfusion medium.

Brief pulses applied to the muscle bundles evoke junction potentials, presumably by stimulating intramuscular nerve branches and terminals.

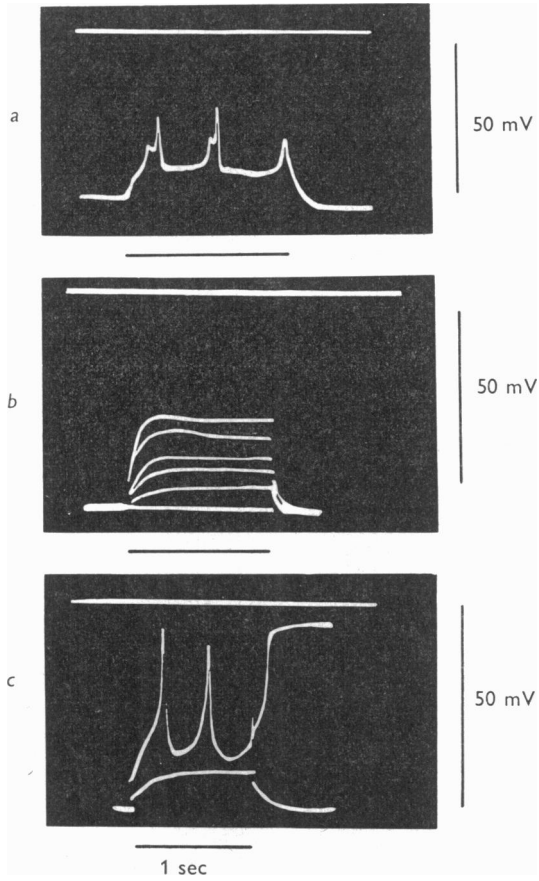

Fig. 3. The effect of 5-HT on responses to direct stimulation of fibres in a small bundle. Upper trace, zero potential; lower trace, membrane potential. Pulse duration, 1 sec. (*a*) 10^{-6} M 5-HT, single stimulus. (*b*) 10^{-8} M 5-HT, 5 responses to stimuli of increasing voltage, superimposed. (*c*) 10^{-6} M 5-HT, same penetration as in (*b*), two responses to stimuli of increasing voltage.

Figure 2 shows the effect of high 5-HT on junction potentials, spike generation and contraction. The junction potential is prolonged. Spikes are fired, which are usually repetitive, and the bundle contracts strongly. While large amplitude junction potentials are observed in low 5-HT, spikes and contraction are very rarely observed, even with strong stimuli. In high 5-HT a remarkable decrease in threshold for contraction was always noted, shorter duration and lower voltage stimuli being required to evoke spikes

and contraction. These results suggest that the critical depolarization required to trigger spike discharge is diminished by 5-HT.

Figure 3 displays the effect of 5-HT (10^{-6} M) when prolonged stimulating pulses were applied. In this case, the muscle was probably stimulated directly by electrical current flow rather than via intramuscular nerves. No junction potential was observed. In high 5-HT, repetitive spikes were

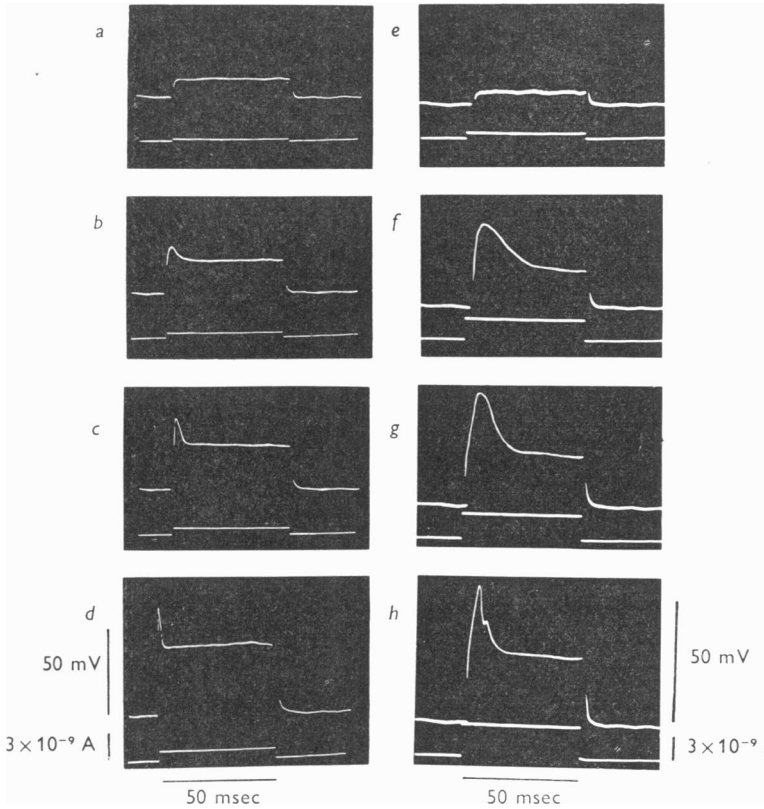

Fig. 4. The effect of 5-HT on the current–voltage relationship in fibres in small bundles. Upper trace, membrane potential; lower trace, stimulus current monitor. (*a*), (*b*), (*c*), (*d*) 10^{-8} M 5-HT; (*e*), (*f*), (*g*), (*h*) 10^{-7} M 5-HT.

fired in response to depolarizing pulses of 1 sec duration. In low 5-HT, strong depolarization failed to evoke spikes. The resting potential was not changed by 5-HT. Again, and more directly, the results suggest that 5-HT may lower the critical depolarization required to fire spikes.

Measurements of the electrical resistance of the cell membrane were successful in six cases. It was difficult to satisfy all the criteria for a valid measurement owing to changes in the electrode properties during the ex-

periment. Successful results appeared to depend particularly on the micropipette itself. Figure 4 shows a typical experiment to determine the current–voltage relationship as a function of 5-HT concentration. The results of the successful experiments indicate that in low 5-HT, the effective membrane resistance, $R_{eff.}$ = 45–60 MΩ and in high 5-HT, $R_{eff.}$ = 23–45 MΩ. The active response recorded at the onset of depolarizing current flow in Fig. 4 is greater in amplitude and longer in duration in high 5-HT. However, in other experiments the amplitude of the active response was greater in low 5-HT. This is in striking contrast to the case where the depolarizing stimuli were applied through external electrodes, in which lowering of threshold was consistently observed.

DISCUSSION

5-HT had little or no effect on the membrane of the innervated, whole muscle. The observed ineffectiveness of 5-HT can be related to previous reports that catch is minimal in the nerve–muscle preparation and that responses to neural stimulation are typically phasic (Twarog, 1960*a*, 1967*a*) if it is assumed that significant quantities of 5-HT are discharged from intramuscular relaxing nerves by spontaneous neural activity as well as by stimulation of the mixed nerve supplying the byssus muscle. If the level of endogenous 5-HT release is high in the innervated preparation, added 5-HT would be relatively ineffective. In the small denervated muscle bundles, where membrane properties are affected by 5-HT concentration, it is assumed that very little endogenous 5-HT is released.

The mechanical properties of the *Mytilus* fibre membrane vary with 5-HT concentration. Penetration of fibres with micro-electrodes is virtually impossible when 5-HT is absent from the bathing medium. It has been noted that mammalian smooth muscle fibres become very difficult to penetrate under certain conditions, particularly when external Ca^{2+} concentrations are increased above normal (H. Kuriyama, personal communication). Shanes (1958) has discussed changes in rigidity of biological and artificial lipid membranes as a function both of temperature and calcification. The 5-HT effect on mechanical properties of the membrane may be direct or related to effects of 5-HT on calcium binding in lipids, as reported by Woolley & Campbell (1960).

Another outstanding action of 5-HT on the fibre membrane is the apparent lowering of the critical depolarization required to trigger spike discharge. Whether depolarization is achieved by neural stimulation or direct stimulation of the muscle membrane, spikes are discharged readily only when 5-HT concentrations are at least 10^{-7} M. A possible interpretation of this increased excitability arises from a speculation that 5-HT reduces the level of intracellular free calcium (Twarog, 1966). Hagiwara

& Nakajima (1966) found that spikes were discharged in a barnacle muscle only when intracellular free calcium levels were reduced below 5×10^{-8} M. In barnacle muscle, the spike depends on currents carried by calcium ions. No one has yet tested whether the *Mytilus* muscle spike depends on Ca^{2+}. However, since the *Mytilus* spike is insensitive to tetrodotoxin (Twarog, 1967*b*), it is reasonable to suppose that it is not a sodium spike, and may well be dependent on Ca^{2+} ion gradients, as in the barnacle.

At 10^{-7} M 5-HT, the effective resistance of the membrane decreased. This is concluded on the assumption that the method permitted accurate measurement of relative values of $R_{eff.}$. The absolute values of $R_{eff.}$ reported here may be in error because a single intracellular electrode was used both for stimulation and recording (Schanne, Kawata, Schäfer & Lavallée, 1966). However, the applied current in these experiments was always below 5×10^{-9} A.

Gerschenfeld & Stefani (1966) reported that 5-HT decreased membrane resistance in molluscan neurones (CILDA cells). While in CILDA neurones, 5-HT was excitatory, and depolarized the membrane, in *Mytilus*, 5-HT did not itself excite or change the membrane potential, although it indeed increased the excitability of the membrane. The experiments of Gerschenfeld & Stefani (1966) clearly indicated an action of 5-HT, on specific 5-HT receptor sites located on the neurone surface in the region of the axon hillock. In *Mytilus* muscle, the action of 5-HT seems quite different. It is possible that 5-HT acts at intracellular sites in *Mytilus*. Born (1962) has shown that 5-HT is able to diffuse into smooth muscle cells.

In conclusion, 5-HT has been shown to increase excitability and decrease membrane resistance in *Mytilus* muscle at concentrations where catch tension is totally abolished. These various effects of 5-HT are not understood and may be mutually unrelated. However, it has been suggested that these results may be produced by an effect of 5-HT on calcium ion binding, both at intracellular sites and at the cell membrane.

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