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EFFECTS OF ACETYLCHOLINE AND 5-HYDROXYTRYPTAMINE ON THE CONTRACTION OF A MOLLUSCAN SMOOTH MUSCLE

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Tension developed by the anterior byssal retractor of *Mytilus* in response to acetylcholine (ACh) decays very slowly after washing off the stimulating agent. 5-Hydroxytryptamine (5-HT) does not block tension development but relaxes the prolonged contraction. When 5-HT is present, tension is maintained only during stimulation (Twarog, 1954; Hoyle & Lowy, 1956). Acetylcholine-blocking agents prevent tension development, but even in high concentration do not affect tension decline in a contracted muscle (Twarog, 1959).

Acetylcholine and 5-HT have been detected in the byssus retractor by bioassay. Cholinesterase activity has been demonstrated (Twarog, 1954). Blaschko & Hope (1957) have found monoamine oxidase activity in the retractor muscle. Spectrophotofluorometric methods have revealed 5-HT in *Mytilus* ganglia (J. H. Welsh & M. Moorhead, unpublished).

Acetylcholine and 5-HT may be mediators released by nerves supplying the byssal retractor muscles. Details of the interaction of ACh and 5-HT on the excitability of the muscle membrane have therefore been studied. The influence of 5-HT and of certain acetylcholine-blocking agents on neural excitation of the muscle has also been examined.

METHODS

The equipment and basic procedure for mechanical and electrical recording and stimulation are described in detail in the preceding paper (Twarog, 1960).

In observations on membrane excitability and contraction maintenance, the muscle was dissected as described by Twarog (1954), teased down to a bundle about 1 mm in diameter and passed through a paraffin-lined slot in the chamber diagrammatically shown in Fig. 1. Tension and electrical potentials were simultaneously recorded on the ink-writer. Total demarcation potential, between the KCl-depolarized and sea-water segments, was 10–15 mV, never reaching the 18–25 mV observed with intact, unteased muscle. This may indicate injury in teasing. However, the potential was stable and bundles had the advantage that small-potential 'spontaneous activity' was virtually absent (Twarog, 1959).

When applying anodal and cathodal polarization only tension was recorded. The whole

muscle, cleaned from nerve, was used in the chamber shown in Fig. 1. At the shell end the chamber was drained and the muscle, suspended in air, made contact with a wick electrode. This, and the large loop of silver–silver-chloride wire in the sea-water chamber served as stimulating electrodes. The polarity of stimulus (e.g. anodal) refers to that of the sea-water chamber. In the nerve–muscle preparation tension was recorded simultaneously with the electrical potential between byssus and the region of nerve entry (Fig. 5).

RESULTS

The interaction of acetylcholine and 5-hydroxytryptamine: typical effects on membrane excitability and maintenance of contraction

When ACh was applied to a muscle previously untreated with drugs, it caused depolarization and development of tension (Fig. 1*a*). Each spike-like potential on the rising phase of depolarization was followed by a

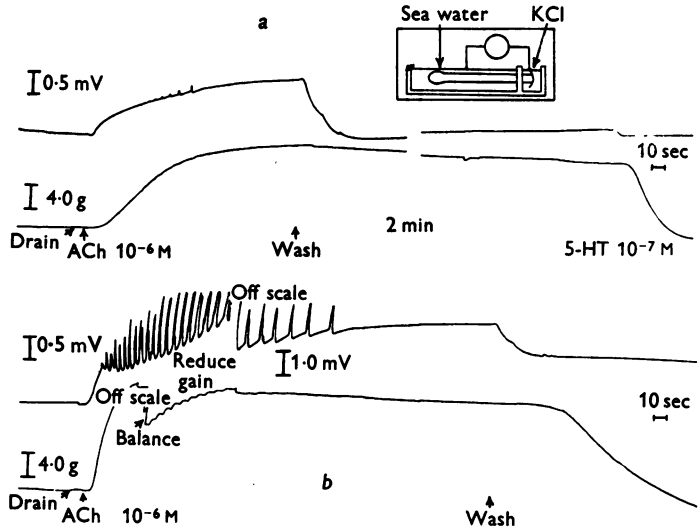

Fig. 1. (Inset) Recording of polarization changes in test segment of anterior byssal retractor muscle, with reference to KCl-depolarized segment. (*a*) Depolarization (upper channel) and contraction (lower channel) with ACh; relaxation by 5-HT. (*b*) ACh effects in the presence of 5-HT; records off scale, rebalanced and amplification reduced in electrical channel.

distinct small increment in tension. After washing, the muscle repolarized while tension remained high. When 5-HT 10^{-7}M was applied, the tension fell abruptly. If the 5-HT 10^{-7}M remained in the bath and ACh was then added (Fig. 1*b*), a striking change was seen. Large spike-like potentials and corresponding tension increments were prominent. The total tension developed was much increased. Tension was sustained after cessation of the rapid potentials until the muscle was washed, when rapid relaxation occurred. Clearly, 5-HT reduces the capacity of the muscle to sustain tension while potentiating electrical activity and tension development.

An interesting example of the relationship between phasic tension development and the maintenance of tension is seen in Fig. 2. This muscle had been left during the night at room temperature and responded to ACh with depolarization and a rhythmic discharge of potentials. Tension was developed and sustained with small tension increments apparent on the crest. (The response resembled that of the 5-HT-treated muscle of Fig. 1*b*.) When 5-HT was applied, as shown in Fig. 2, the rhythmic electrical activity was only slightly modified (increased amplitude, decreased fre-

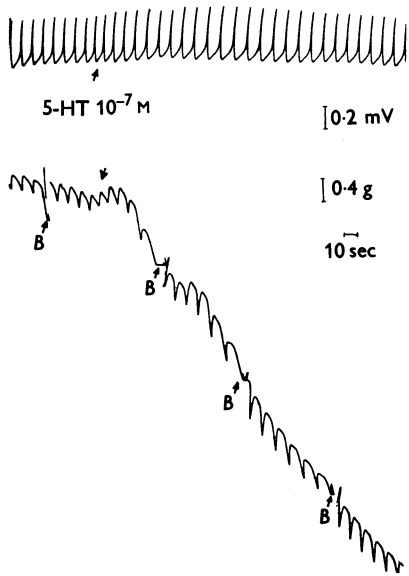

Fig. 2

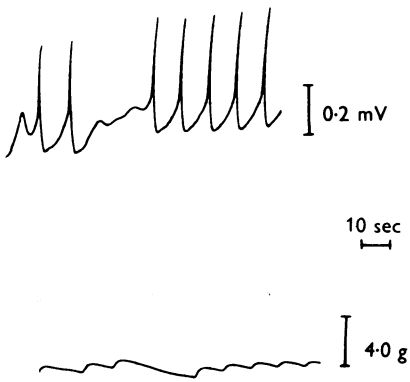

Fig. 3

Fig. 2. Electrical activity (above) and tension (below) in a rhythmically contracting muscle. Relaxation of maintained tension by 5-HT, off scale, rebalanced (*B*) and records appropriately aligned. Note constancy of rhythmical activity during relaxation.

Fig. 3. Electrical activity (above) and tension (below) in a rhythmically contracting muscle. Note failure to develop tension in absence of spike-like potential, lack of relaxation during cessation of discharge.

quency) and rhythmic contractions continued, while the muscle rapidly and fully relaxed. In Fig. 3 the same preparation as in Fig. 2 is seen during the maintained contraction (before 5-HT). Electrical activity was occasionally irregular. A small, rounded electrical potential appeared at time intervals appropriate to the original rhythm, when the spike was absent, but no tension increment accompanied this potential. It is noteworthy that relaxation following spike failure was negligible compared with the relaxation in Fig. 2 which occurred during continuous spike activity.

Effects of anodal and cathodal polarization on development and maintenance of tension

Sensitivity to anodal and cathodal polarization differed during spike-coupled rhythmic tension changes and maintained tension, as is seen in Fig. 4. Here an anodal direct current pulse had no effect when applied during the last stages of tension decay following a long cathodal pulse. A long cathodal pulse at this time led to a prolonged contraction. Brief repetitive cathodal pulses had no effect, while brief anodal repetitive pulses of the same voltage led to relaxation after a small tension increase. (The effectiveness of brief anodal pulses was noted by Fletcher (1937), and may be significant

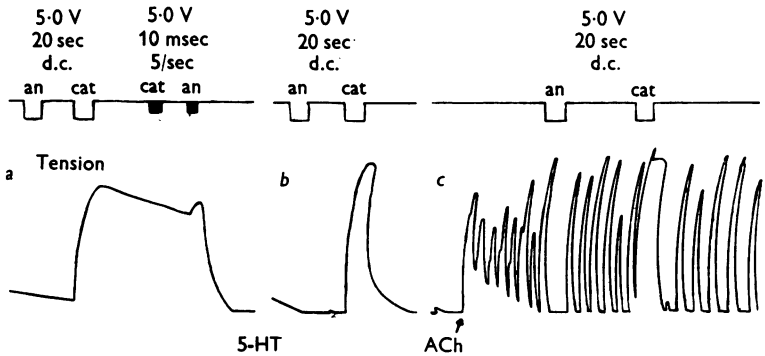

Fig. 4. Effects of anodal (an) and cathodal (cat) polarization on tension. (*a*) Muscle in last stages of maintained contraction. Anodal and cathodal direct current, single long pulses, then brief repetitive pulses. (*b*) Muscles relaxed in 5-HT; long anodal and cathodal pulses. (*c*) Muscle rhythmically contracting in ACh plus 5-HT; long anodal and cathodal pulses.

in synchronizing responses.) A cathodal pulse again led to prolonged tension in this preparation, which was relaxed by 5-HT (not shown). In the presence of 5-HT, a long anodal pulse was again without effect, while a cathodal pulse led to a larger tension increase which relaxed at the cessation of stimulation. In the continued presence of 5-HT, the response to ACh was a series of rhythmic contractions. In the rhythmically active muscle, anodal direct-current pulses inhibited contraction and decreased the tension level, while cathodal polarization led to a contraction which relaxed at the end of stimulation.

Effects of acetylcholine-blocking agents on neural excitation of the muscle

Stimuli of 1 msec duration were applied through the nerve at the rate of 1/sec, as in Fig. 5. The upper, control, record showed facilitation of contraction with repetition of identical stimuli. After soaking the muscle for 3 min in 10^{-3}M banthine, the contractile response was blocked and the

electrical response became smaller and rounded in shape. Other ACh-blocking agents, including atropine and benzoquinonium, similarly altered the large spike-like potential and blocked the contractile response to neural excitation at concentrations between 10^{-4} and 10^{-3}M. In concentrations of 5-HT up to 10^{-4}M, the electrical response was not altered nor was contraction blocked, although an increase in threshold was noted in some experiments.

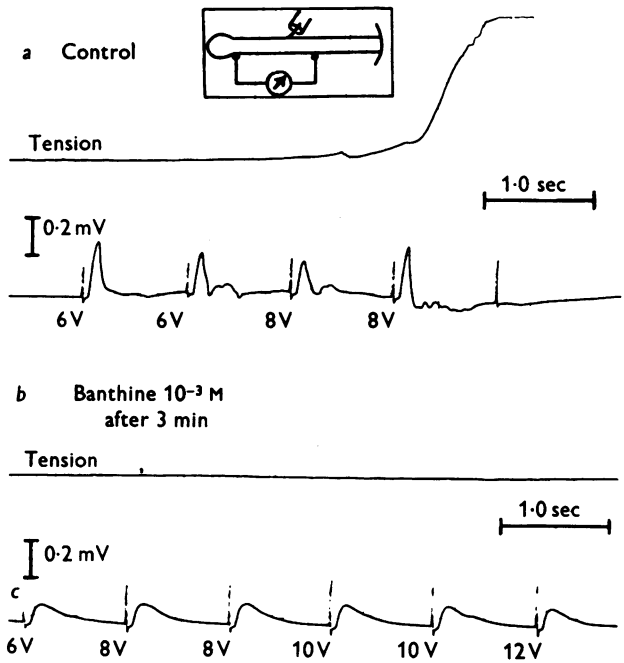

Fig. 5. (*a*) Untreated muscle; electrical response below, tension above; note facilitation with 8 V shock. (*b*) After banthine 10^{-3}M; tension (above) blocked; electrical response (below, reduced) prolonged. Inset, placement of stimulating and recording electrodes.

DISCUSSION

Block of neural excitation by ACh-blocking agents, as observed here, supports a hypothesis of excitatory transmission by ACh. It has been argued by Hoyle & Lowy (1956) that 5-HT cannot be a transmitter, since its action was not easily reversible in their experiments. In the present study the effects of concentrations of 5-HT which fully relax the muscle were reversed within minutes by washing. The apparent discrepancy may be due to the higher concentrations used by Hoyle & Lowy. Enzymic destruction of 5-HT in the byssus retractor has been demonstrated by Blaschko & Milton (1959).

Rhythmic contractions in combined ACh and 5-HT have been seen in

other molluscan smooth muscles (Hill, 1958; Fänge & Mattisson, 1958). In the present study rhythmic spike-like potentials preceded rhythmic contractions. Anodal polarization inhibited these contractions. In guinea-pig smooth muscle, spike discharge and tension are suppressed by anodal polarization (Bülbring, 1956). It appears probable that rhythmic contractions of the byssus retractor arise from electrical activity of the muscle membrane.

The prolonged contraction after ACh is apparently not associated with electrical activity (this paper and Twarog, 1960), nor was prolonged contraction relaxed by anodal polarization. This suggests that the elements which maintain tension may be independent of membrane polarization and electrical activity, once tension has been developed. Johnson, Kahn & Szent-Györgyi (1959) suggested that the tension-developing and -maintaining systems have separate molecular bases.

The action of 5-HT somewhat resembles β inhibition in crustaceans. As in studies by Hoyle & Wiersma (1958) on crustacean muscle, large mechanical effects accompanied very small changes in membrane polarization. According to Hoyle & Wiersma an inhibitory transmitter might block excitation–contraction coupling or could cause a change in permeability to ions important in contraction, such as Ca^{2+}. If tension maintenance is independent of the membrane activity which leads to tension development, a relaxing mechanism specific to the tension-maintaining system must exist. If continued activation is not involved in tension maintenance, then uncoupling is a possible but unnecessary postulate.

SUMMARY

1. Relaxing concentrations of 5-hydroxytryptamine (10^{-7} M) altered the response of the byssus retractor of *Mytilus* to acetylcholine. In the presence of 5-hydroxytryptamine, acetylcholine evoked a succession of spike-like electrical discharges. Total tension developed was greater than in the absence of 5-hydroxytryptamine but the contraction was not sustained following removal of acetylcholine.

2. Anodal polarization inhibited rhythmic contractions but did not accelerate the slow decline of tension after acetylcholine.

3. Acetylcholine-blocking agents (banthine, atropine and benzoquinonium, 10^{-4}–10^{-3} M) prevented the contractile response to neural stimulation and reduced the electrical response. 5-Hydroxytryptamine (as high as 10^{-4} M) did not interfere with neural excitation.

4. While firing of spike-like potentials appears necessary for tension development, tension following stimulation may be maintained by non-discharging elements. Possible mechanisms of relaxation by 5-hydroxytryptamine are discussed in this connexion.

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