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# EFFECTS OF 5-HYDROXYTRYPTAMINE ON ACTION POTENTIALS AND ON CONTRACTILE FORCE IN THE VENTRICLE OF *DOLABELLA AURICULARIA*

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SUMMARY

1. Concentrations of 5-hydroxytryptamine from $10^{-8}$ to $10^{-5}$ M had the effect of starting quiescent ventricles, with a slow slight depolarization followed by the onset of beating, with hyperpolarization between beats.

2. Concentrations of 5-hydroxytryptamine from $10^{-7}$ to $10^{-3}$ M had the effect of prolonging the cardiac action potential and increasing force. Over the entire range, an increase in concentration led to an increase in the duration and amplitude of the plateau phase of the action potential, and an increase in force of contraction.

## INTRODUCTION

There is substantial evidence (reviewed, Hill & Welsh, 1966) that the cardioregulatory neurohumours released by the cardiac nerve endings in molluscan hearts include an inhibitory acetylcholine-like substance and an excitatory 5-hydroxytryptamine-like substance. Since fluorescence methods (Cardot, 1971) have demonstrated the presence of 5-hydroxytryptamine (5-HT) in nerve fibres of a number of molluscan hearts, 5-HT itself may well be the cardio-excitatory transmitter. Many molluscan hearts have low thresholds for the application of acetylcholine (ACh) or 5-HT (Hill & Welsh, 1966), and presumably have specific ACh or 5-HT receptor sites, since the thresholds for ACh or 5-HT are lower than for series of analogues (Welsh & Taub, 1951: Greenberg, 1960, 1970). Although 5-HT evidently affects excitability (Hill & Thibault, 1968), it also has a profound inotropic effect on force of gastropod ventricles (Hill, 1958). Since tension in the myocardium of *Dolabella* is directly related to the duration of the cardiac action potential (Nomura, 1963), it is important to consider whether or not the strongly positive inotropic effect of 5-HT is due to an effect on the duration of the plateau of the cardiac action potential. Such prolongation of the depolarization might be expected to allow more time for the development of contraction by prolonging the entry of $Ca^{2+}$ into the myoplasm, thereby enhancing the degree of excitation–contraction coupling (Nomura, 1965).

## MATERIALS AND METHODS

The ventricle of the Hawaiian *Dolabella* (Family, Aplysiidae; subfamily, Dolabellinae) was used. Following Engel (1942) the species is *D. auricularia* Solander, 1786 = *D. scapula* Martyn, 1784, an invalid name (Marcus, 1965). The animal is readily available (Kay, 1964) and of large size, and the ventricle can be set up as a stretched tube suitable for use in a sucrose-gap apparatus.

Isolated ventricles were prepared from specimens that had been collected from the reef off Ala Moana Park, Honolulu, and kept in tanks of running ocean, or well, sea water, where they remained active and appeared normal for the period during which they were retained. Entire hearts were dissected out rapidly, and then pinned out in dilute methylene blue solution in sea water, until the chambers could be readily distinguished. Ligatures were then tied tightly around the junctions between ventricle and cristae aortae, and ventricle and auricle, before cutting away cristae aortae and auricle. Ventricles were then set up in a single sucrose-gap apparatus similar to that described by Berger & Barr (1969), with rubber membranes between the compartments. At the beginning of each experiment, the isolated ventricle was set up horizontally in the sucrose-gap apparatus, with the auricular end of the ventricle in the (left-hand) compartment used for depolarizing with KCl, and the aortic end of the ventricle in the (right-hand) compartment used for superfusion with solutions of pharmacological agents. Both end-compartments were initially perfused with filtered ocean sea water, while the central compartment was perfused with 0·75 M sucrose solution. After the sucrose had flowed for at least 30 min in order to allow time for it to penetrate the preparation, the gap potential was measured as a control value in each experiment. Subsequently, the ventricle was depolarized in one end-compartment by perfusion of the compartment with 0·5 M-KCl. The other end-compartment was perfused with sea water, or sea-water solutions of acetylcholine iodide (Sigma) or 5-hydroxytryptamine creatinine sulphate (Sigma). Gap potential difference was recorded by sintered silver–silver chloride pellet electrodes in the two end-compartments. Tension at either end was recorded with a Harvard Apparatus Co. Model 363 Isometric Force Transducer. Up to five display traces were photographed from the face of a Tektronix Model 5103N oscilloscope.

## RESULTS

The sucrose-gap method proved to be a useful technique for recording from contracting gastropod ventricles. The trabecular architecture is so loose that the amplitude of movement during each contraction leads to greater difficulty in keeping a microelectrode inserted during a beat than is the case in the thinner-walled bivalve hearts. With the sucrose-gap technique, it is possible to carry out lengthy series of experiments without interruption.

Initial control gap potential differences of 3–6 mV were observed, probably attributable to a difference in the base tension applied to the ends of the ventricle in the right and left compartments; these potential differences were backed off with a balancing voltage before depolarization of one end.

In the whole series of experiments the maximum resting potential measured was 40 mV. That may be compared to a mean resting potential value of 45 mV and

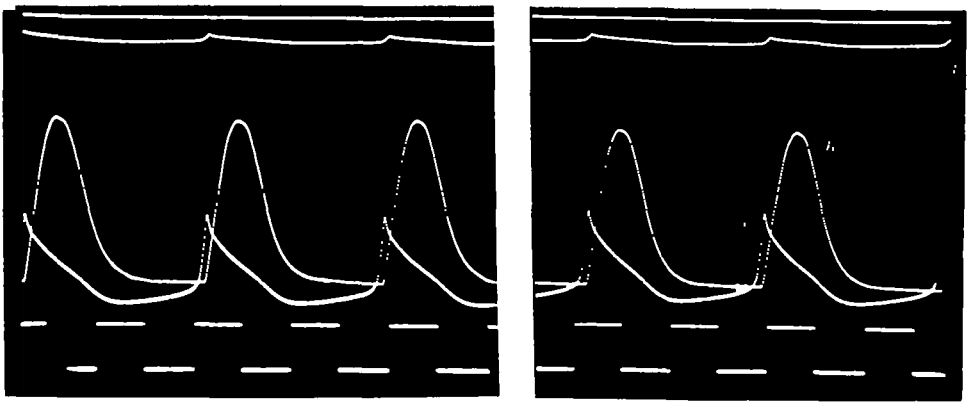

Fig. 1. Representative record of electrical and mechanical activity of a spontaneously beating ventricle of *Dolabella auricularia*. (*a*) Monitor of gap potential difference (PD). The upper line represents zero potential difference across the sucrose gap; lower line, potential difference after depolarization of one end of the ventricle with KCl; maximum value, 33 mV. (*b*) Force of contractions. (*c*) Cardiac action potentials. Time signal 0·5 Hz in this and all succeeding figures (i.e. each cycle takes 2 sec but each square wave has a 1 sec pulse duration). The height of each square wave corresponds to 50 mV for (*a*) but to 5 mV for (*c*) and 250 mg for (*b*). The same system of calibration is used in all figures.

maximum value of 75 mV, recorded from the ventricle of *Dolabella* by Kuwasawa (1967) using a micro-electrode technique. Fig. 1 is a representative example of the conventions employed in recording electrical and mechanical activity simultaneously from isolated ventricles of *Dolabella auricularia*. Gap potential was monitored continuously at low gain in order to keep a record of resting potential on screen, since it was desirable to display action potentials at a greater amplification. The deflexions on trace *c* are spontaneous action potentials, while those on trace *b* are spontaneous contractions.

### *Effect of 5-hydroxytryptamine in starting quiescent ventricles*

Gastropod ventricles *in vitro* are dependent on internal prefusion for the appearance of automaticity (Hill & Welsh, 1966; Hill & Irisawa, 1967). Presumably the effective factor is longitudinal stretch of cardiac muscle fibres. In this study, the isolated ventricles were deliberately set up under just enough longitudinal tension so that slight mechanical contractions could be recorded, and spontaneous automaticity did not always arise. Non-beating ventricles were superfused with $10^{-8}$ to $10^{-5}$ M 5-HT in 15 trials. Four of those ventricles had remained quiescent when first depolarized, and 11 had spontaneously stopped beating. The typical effect of 5-HT application (Fig. 2) was a slow depolarization (0·8–17 mV) followed by the onset of beating, after which the 'resting potential' was hyperpolarized. Qualitatively, slight depolarization by 5-HT was as effective as a larger depolarization in restoring activity.

### *Effect of 5-hydroxytryptamine on duration of the plateau phase of the cardiac action potential, and on force of contraction*

The threshold concentration of 5-HT for an effect on actively beating ventricles was $10^{-8}$ M. Application of $10^{-5}$ M 5-HT (Fig. 3) caused the appearance of a plateau

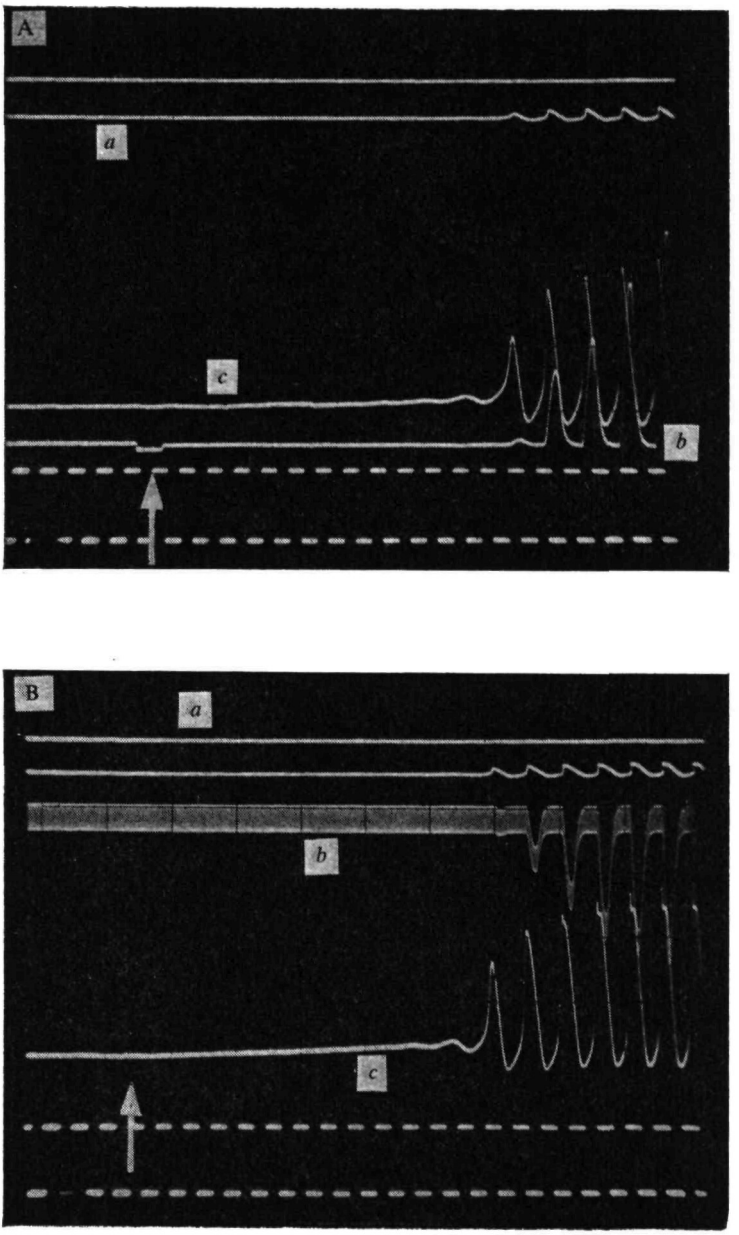


Fig. 2. (A) Superfusion with $10^{-7}$ M 5-HT at the arrow. (B) Superfusion with $10^{-6}$ M 5-HT at the arrow. Calibration signal: for (*a*) 50 mV, for (*b*) 250 mg in (A) and 2·5 g in (B), for (*c*) 10 mV. Increase in force is an upward deflexion in (A) and a downward deflexion in (B).

on the action potential and an increase in force with the next beat. As the duration and amplitude of the plateau waxed and waned, force also waxed and waned. The effect of 5-HT on the plateau is shown more clearly in the next illustration (Fig. 4). Superfusion with $10^{-5}$ M 5-HT caused a plateau on the cardiac action potential and force attained in each beat was 750 mg (Fig. 4A). After washing out the 5-HT the platea

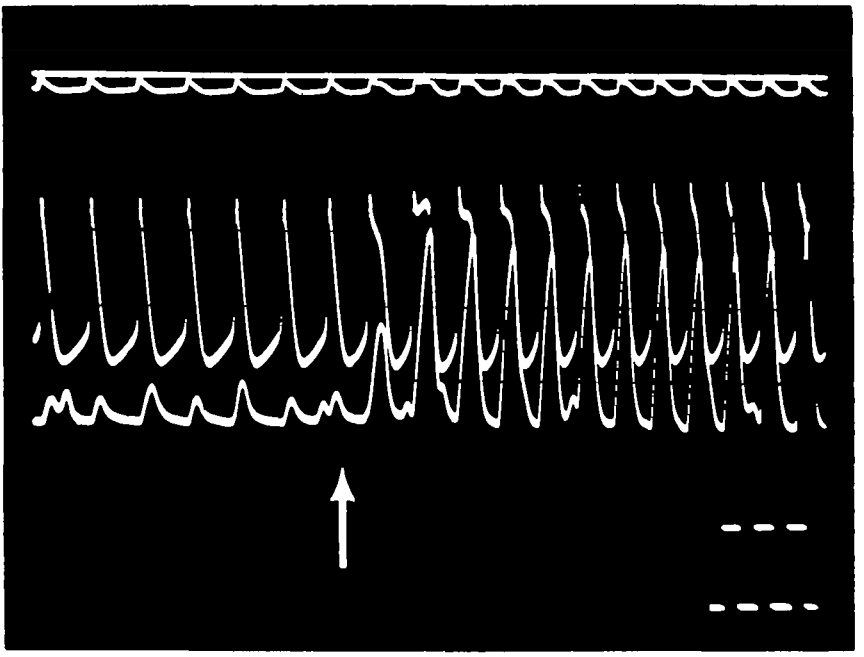

Fig. 3. Superfusion with $10^{-5}$ M 5-HT at the arrow. Upper pair of traces; gap potential monitor; calibration 50 mV. Middle trace: cardiac action potentials, note onset of plateau phase after superfusion begins; calibration, 5 mV. Lower trace: force (note – after onset of plateau phase – force is correlated with duration of plateau). Calibration, 500 mg.

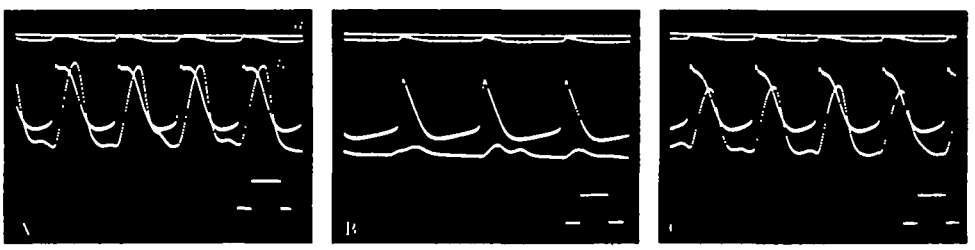

Fig. 4. Effect of 5-HT on the plateau of the cardiac action potential. (A) In $10^{-5}$ M 5-HT. (B) In sea water. (C) in $10^{-5}$ M 5-HT. (*a*) Gap potential monitor; maximum PD, 32 mV; calibration, 50 mV. (*b*) Force of contractions; calibration, 250 mg. (*c*) Cardiac action potentials; calibration 5 mV.

phase was abolished and force was reduced by nine-tenths to 80 mg (Fig. 4B). Superfusion with $10^{-5}$ M 5-HT then restored plateau and force, although not to as marked a degree as before (Fig. 4C).

At a higher concentration of 5-HT the increased duration of the cardiac action potential was accompanied by still greater maximum force. A ventricle which developed 2·5 g force in sea water developed 7·5 g force in $10^{-4}$ M 5-HT (Fig. 5A). This was accompanied by the appearance of a plateau phase in the action potential (Fig. 5B).

Over a range of concentrations of 5-HT it can be seen that the action of a higher level of 5-HT is to prolong the action potential and increase force. In one particular experiment (Fig. 6) the ventricle, contracting in $10^{-8}$ M 5-HT, had action potentials

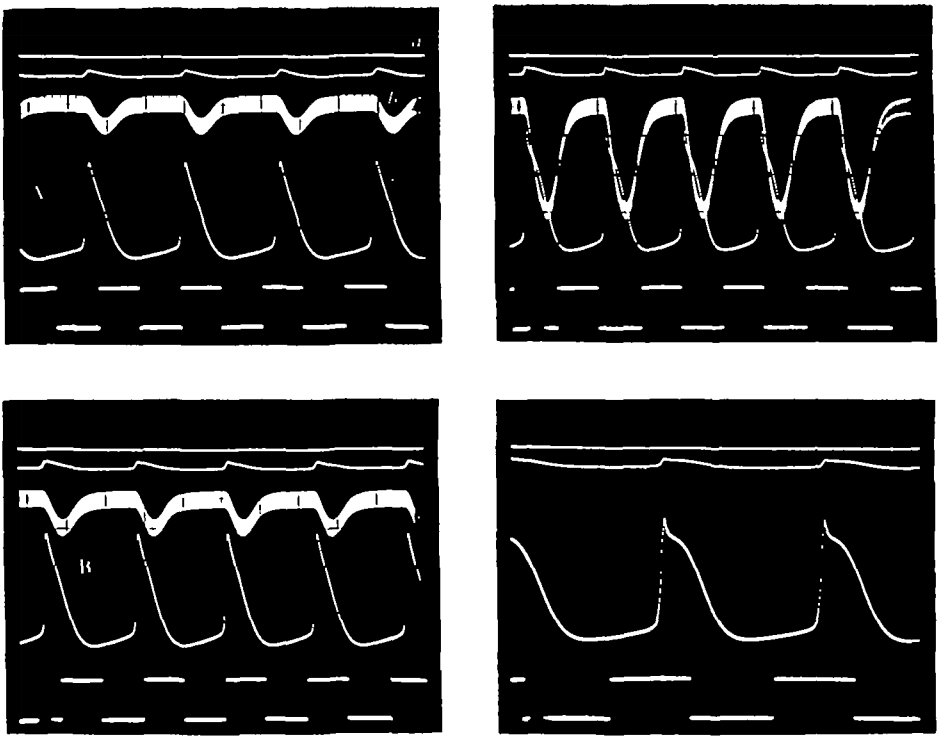

Fig. 5. Effect of $10^{-4}$ M 5-HT on the plateau of the cardiac action potential. (Force downward.)

(A) First block: spontaneous beating in sea water. Second block: superfusion with $10^{-4}$ M 5-HT. (*a*) Gap potential monitor: calibration, 50 mV; maximum PD, 38 mV. (*b*) Force of contractions; calibration, 2·5 g. (*c*) Cardiac action potentials; calibration, 10 mV.

(B) A repetition of A but with the tension record shut off to avoid obscuring the form of the action potential in the second block.

without a differentiated plateau phase (Fig. 6A), and developed 825 mg active force with each beat. Superfusion with $10^{-7}$ M 5-HT lengthened the cardiac action potential by 15% (Fig. 6B) and the force increased to 2·08 g. Superfusion with 5-HT concentrations increasing from $10^{-6}$ to $10^{-4}$ M led to progressive lengthening of the plateau phase and increase in force of contraction (Fig. 6C, D, E) up to 8·25 g. A dramatic effect was obtained by increasing the concentration to $10^{-3}$ M. The plateau developed into a second peak, higher than the spike of the cardiac action potential, and the ventricle became depolarized by 7 mV (Fig. 6F). Active force increased to 12·5 g but there was no contracture. Over the entire range from $10^{-8}$ to $10^{-3}$ M 5-HT, there was a marked increase in force without concomitant increase in spike height (Fig. 7).

## DISCUSSION

The sucrose-gap method is useful as an extracellular method of approximating membrane potential values. The values were obviously attenuated, since the maximum resting potential value was 40 mV. Attenuation is a problem to be expected with a sucrose-gap method, and 50% attenuation with cardiac muscle is not unusual (Harrington & Johnson, 1973).

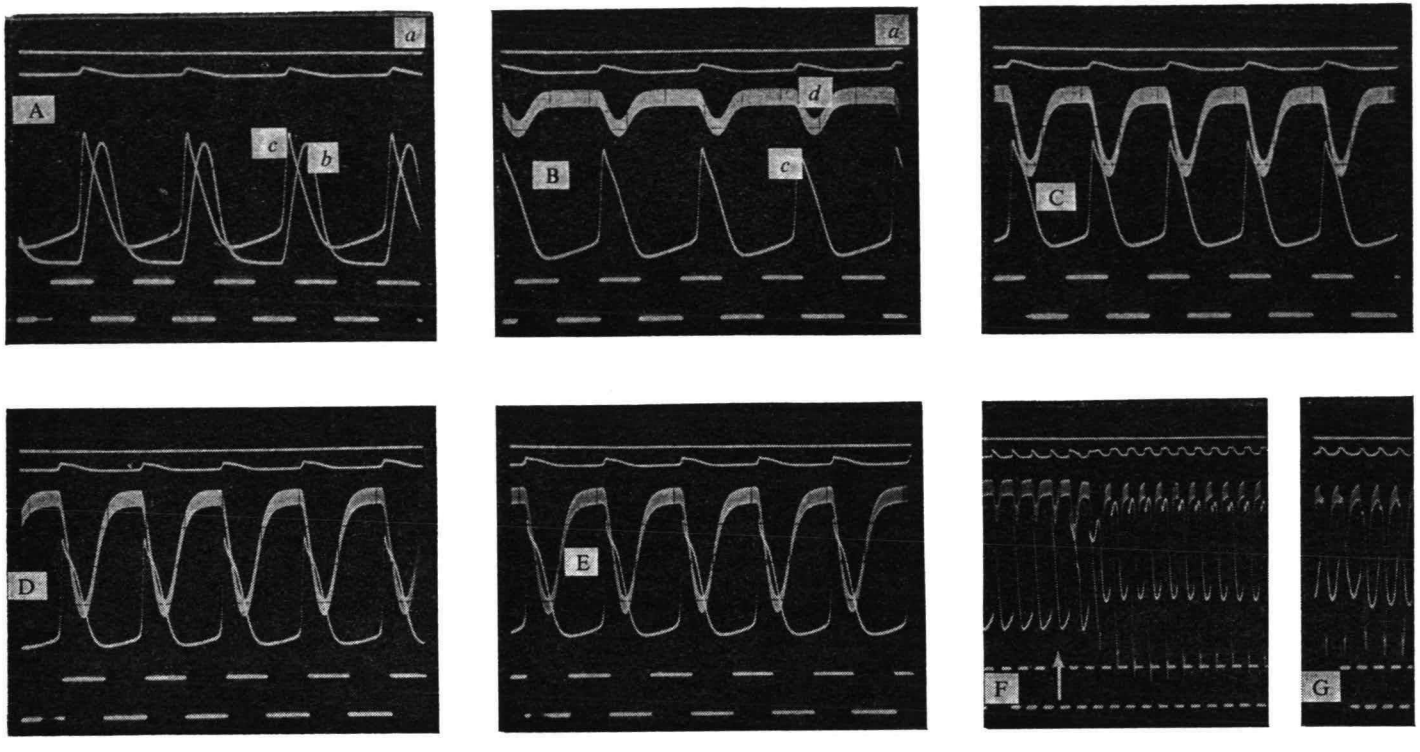


Fig. 6. (A) Effect of superfusion of one end of the ventricle with $10^{-6}$ M 5-HT; other end depolarized. (*a*) Gap potential monitor: maximum PD 38 mV; calibration 50 mV. (*b*) Force of contractions; calibration, 250 mg. (*c*) Cardiac action potentials; calibration, 10 mV.

(B) Superfusion of one end of the ventricle with $10^{-7}$ M 5-HT in sea water. (*d*) Force of contractions; calibration, 2·5 gm; increase in force-downward deflexion.

(C) Superfusion with $10^{-6}$ M 5-HT in sea water.

(D) Superfusion with $10^{-5}$ M 5-HT (after adaptation).

(E) $10^{-4}$ M 5-HT.

(F) $10^{-3}$ M 5-HT applied at the arrow (resting potential, 22 mV).

(G) After 2 min in $10^{-3}$ M 5-HT.

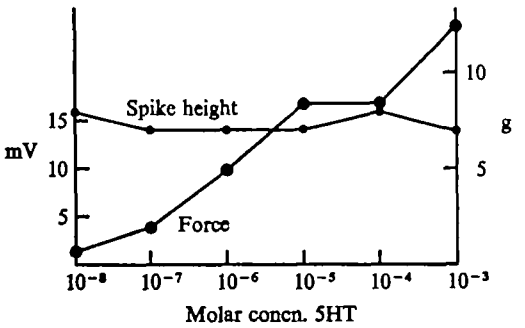


Fig. 7. Relation of spike height to force developed at various concentrations of 5-HT.

In a number of studies it has been shown that vigorously beating gastropod ventricles possess an action potential with an initial spike followed by a plateau (e.g. Ripplinger & Ripplinger, 1973). Suction electrode recording from the ventricle of *Rapana thomasiana* has been used to show that reduction in perfusion pressure leads to loss of the differentiation of the cardiac action potential into spike and plateau phases (Hill & Irisawa, 1967). This loss of differentiation at reduced perfusion pressures was accompanied by reduced force.

Nomura (1963) used micro-electrodes to record the intracellular action potential from ventricular muscle fibres of *Dolabella auricula* (= *D. auricularia*) in a study of the relation between duration of action potential and degree of contraction. He found action potentials consisting of a spike and a plateau, and preceded by a slow diastolic depolarization. There was a linear relationship between magnitude of resting potential and amplitude of action potential, but no overshoot was recorded. He also showed that there was a relationship between the stretch of the cardiac muscle fibres and the duration of the plateau of the action potential, and that increased duration of the plateau was accompanied by an increase in force.

The idea of a precise link between the area of the action potential and the degree of activation has been clarified for muscle in general by Sandow (1965) in his formulation of excitation–contraction coupling. In his hypothesis the mechanical effectiveness of an action potential depends on the area bounded (in PD) by the levels of mechanical threshold and mechanical saturation, and (in time) by the rise and fall of the action potential between those levels. Thus, according to current concepts of excitation–contraction coupling, if a prolonged plateau phase of the cardiac action potential maintains the PD over mechanical threshold for a longer period of time, this should increase the mechanically effective area and increase the amount of activation of the contractile apparatus. A relationship between duration of action potential and force is ordinarily not evident in spontaneously beating isolated gastropod hearts, but may be demonstrated by techniques, such as stretching or treatment with 5-HT, which prolong the duration of the cardiac action potential.

### *Effect of 5-hydroxytryptamine on gastropod hearts*

The threshold for an effect of superfusion with 5-HT was found to be at $10^{-8}$ M quiescent or actively beating isolated ventricles of *Dolabella auricularia*). This corresponds to the threshold for an effect of 5-HT on a beating ventricle of *Aplysia*

*Dolactylomela* (Hill, 1958) or a quiescent ventricle of *Aplysia fasciata* (Hill, 1964). The effect of 5-HT in starting quiescent ventricles of *Dolabella auricularia* is rather similar to the effects of 5-HT on the ventricle of a bivalve, *Mytilus edulis*. Irisawa, Wilkens & Greenberg (1973) found that 5-HT 'could induce or augment rhythmical activity while producing only a small depolarization'. $10^{-6}$ 5-HT led to the appearance of bursts of spikes in a *Mytilus edulis* ventricle previously unresponsive to applied current pulses, but had a small effect on membrane conductance.

The observation that the increased duration of the cardiac action potential of the ventricle of *Dolabella auricularia* (induced by application of 5-HT) is accompanied by an increase in force agrees with Nomura's demonstration of a relation between the duration of the cardiac action potential and force of contraction. This leads one to the conclusion that 5-HT may have two sites of action on the gastropod ventricle. Kuwasawa has observed (personal communication) that after application of 5-HT, EPSP's are transformed into action potentials. Such an observation is in accordance with the evidence that 5-HT may be the excitatory neurotransmitter for molluscan hearts (Hill & Welsh, 1966). However, the diffuse nature of the innervation of gastropod hearts (Baxter & Nisbet, 1963) has led to the supposition that excitatory axons may function by releasing neurohumours in the vicinity of cardiac muscle fibres. If 5-HT is released in this manner, it might also be available to affect E–C coupling by affecting the duration of the action potential. Thus the increased force observed, when the duration of the action potential was prolonged by 5-HT, might be ascribed to a prolongation of the time available for the entry of $Ca^{2+}$ into the myoplasm (Sandow, 1965).

E–C coupling may indeed be mediated by $Ca^{2+}$ in gastropod hearts. Nomura (1965) has shown that potassium contracture of cardiac muscle of *Dolabella* is dependent on the calcium content of the bathing medium, to the extent of being completely eliminated in calcium-free fluid. He concluded that it was probable that entry of calcium into the muscle fibres was required for E–C coupling. Thus it may well be that 5-HT, either as a neuro-humour (Welsh, 1957) or as a local hormone (S.-Rózsa & Zs.-Nagy, 1967), controls the force of the cardiac contraction by controlling E–C coupling.

However, 5-HT would appear to have opposite effects on the ventricles of *Dolabella* and *Modiolus demissus*, a bivalve. Wilkens & Greenberg (1973) found that 5-HT may suppress the pacemaker of the *Modiolus* heart, but it initiates beating in quiescent *Dolabella* ventricles; they found 5-HT might interfere with $Ca^{2+}$ flux associated with a $Ca^{2+}$ spike, whereas I suggest 5-HT may promote $Ca^{2+}$ flux associated with E–C coupling. The difference in mode of action is to be expected, since $10^{-8}$ M 5-HT inhibits the heart of *Modiolus demussus* but excite the ventricle of the heart of *Dolabella*.

In Fig. 6 it may be noted that although $10^{-4}$ M 5-HT (E) lengthened the action potential more than $10^{-5}$ M 5-HT (D), it did not produce much more force. Similarly, lengthening of a shortened action potential in mammalian heart muscle beyond a limiting value may not further increase force (Morad & Trautwein, 1968). However, $10^{-3}$ M 5-HT (F), which induces a hump of further depolarization just after the spike, did markedly increase force. This again is consistent with the observation of Morad and Trautwein that most of the E–C coupling may be accomplished by calcium ion release within the first 100 msec of the AP, although the entire length of the plateau may govern entry of calcium into the myoplasm from the extracellular compartment

(Morad & Goldman, 1973). Therefore, it may be suggested that in both taxa, cardiac muscle has evolved with a spike-and-plateau waveform, because such a waveform is adapted to providing control of force through regulation of the duration of the AP.

## CONCLUSION

The effect of 5-HT from $10^{-7}$ to $10^{-3}$ M is to increase the duration of the plateau of the cardiac action potential with a consequent increase in force. If the 5-HT receptors resemble A-receptors of neurones (Gerschenfeld, 1973) this could indicate an increase in membrane permeability to $Na^+$, with the consequent depolarization leading to entry of $Ca^{2+}$-activator. However, there remains the possibility that 5-HT may simply increase $Ca^{2+}$ current.

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