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EFFECTS OF ACETYLCHOLINE ON RESTING AND ACTION POTENTIALS, AND ON CONTRACTILE FORCE IN THE VENTRICLE OF *DOLABELLA AURICULARIA*

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SUMMARY

Acetylcholine (2×10^{-8} to 2×10^{-3} M) depolarized the isolated ventricle of *Dolabella auricularia*. The depolarization was accompanied by a negatively inotropic effect from 2×10^{-8} M to 2×10^{-4} M, and by a positively tonotropic effect from 2×10^{-4} M to 2×10^{-3} M. The interaction of acetylcholine and 5-hydroxytryptamine was studied by artificially prolonging the plateau of the cardiac action potential by treatment with 5-HT. Acetylcholine had the effect of shortening the plateau established by 5-hydroxytryptamine. Force was reduced correspondingly.

INTRODUCTION

Pharmacological experiments on the functions of the innervation of molluscan hearts suggest that the inhibitory cardioregulatory chemotransmitter may be an acetylcholine-like substance. Since the earlier evidence was reviewed (Hill & Welsh, 1966) it has been found that in additional species of Mollusca, acetylcholine (ACh) application inhibits the ventricle, and ACh-antagonists block the inhibitory effects of the cardiac nerves. For instance, Phillis (1966) has shown that pretreatment with 10^{-5} M Mytolon both prevents inhibition of the ventricle of *Tapes watlingi* following application of ACh and blocks the inhibitory effects on the ventricle of stimulation of the nerves to an isolated heart. Similarly S. Rózsa & Zs.-Nagy (1967) have shown that the inhibitory effect of ACh on the isolated heart of *Lymnaea stagnalis* can be blocked by pretreatment with 10^{-5} M Mytolon, as can the inhibitory effect of an agent extracted from the heart. MacKay & Gelperin (1972) found that Mytolon blocked the inhibitory effect upon the ventricle of stimulation of the visceral nerve of *Limax maximus*. Most studies have used mechanical recording from isolated ventricles. Since it is now possible to adapt the sucrose gap technique to entire molluscan ventricles (Wilkens & Greenberg, 1973), the inotropic effects (effects on force) of acetylcholine may be examined simultaneously with effects on cardiac resting and action potentials.

Although gastropod hearts have been frequently used for electrophysiological studies there has been no direct study of the basis of the triphasic concentration–action

curve for acetylcholine (Hill, 1958; Hill, 1964; Hill & Thibault, 1968). In discussion of molluscan hearts, there is a tendency to think of the triphasic action of ACh as inconstantly excitatory at very low concentrations, inhibitory at intermediate concentrations, and excitatory again at very high concentrations. On this basis, one might predict that ACh should be depolarizing at very low concentrations, hyperpolarizing at intermediate concentrations, and depolarizing again at high concentrations. However, my experiments with gastropod hearts have indicated predominantly inotropic effects, which might be mediated either through an effect on excitation–contraction coupling or through changes in the parameters of the action potential that govern the duration of the active state.

METHODS

The methods used have been reported previously (Hill, 1974). Specimens of *Dolabella auricularia* Solander, 1786, were obtained from the reef off Ala Moana beach park in Honolulu. Acetylcholine iodide and 5-hydroxytryptamine creatinine sulphate (5-HT) were obtained from the Sigma Chemical Company.

RESULTS

Each spontaneously beating ventricle had a threshold for acetylcholine of around 2×10^{-8} M. A representative series of experiments on one ventricle appears in Figs. 1 and 2. After each superfusion with ACh solution, the compartment was washed with sea water until there was no further effect of washing, before superfusion with the next solution in an ascending series of concentrations. The experiments were done in the order in which the records are arranged, and the left-hand records in C, D, E of Fig. 1 and A, B, C of Fig. 2 are therefore controls. Although some desensitization and lack of reversibility is evident, the trend was clearly to increased depolarization with increased concentration, from 2×10^{-8} M to 2×10^{-3} M. The depolarizations reported in the legends to the figures are nominal, since it is doubtful whether the gap PD reached the value of the full resting potential, but they are consistent within the set of observations.

Action potentials continued to appear from the reduced level of resting potential at 2×10^{-5} M ACh, but spike height was reduced from 11 to 7 mV. At 2×10^{-4} M (Fig. 2B) superfusion with ACh depolarized by 17 mV and a contracture was induced with onset at 8·4 mV depolarization. After 6 min the preparation had repolarized by 4 mV, and beating at reduced force resumed. Peak force was reduced by 66% from the

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Fig. 1. Time calibration, 0·5 Hz. All other calibrations are given in terms of the amplitude of the time signal. *a*, Gap PD; calibration, 50 mV; max. 35 mV; *b*, Force; calibration, 250 mg; *c*, Cardiac action potentials; calibration, 5 mV; *d*, Tension at depolarized end; calibration, 2·5 g. (A) Depolarization of one end of a ventricle with 0·5 M-KCl. (B) After a 3 min interval; beating begins. (C) Superfusion of the non-depolarized end of the ventricle with 2×10^{-8} M AChI in seawater, for 13 min between first and second set of recordings. Slight reversible depolarization accompanied by reversible diminution in peak force (by 15%). (D) Superfusion with 2×10^{-7} M AChI for 2 min between first and second sets of recordings. Slight depolarization, marked diminution in peak force (by 35%) and slight negative chronotropic effect. (E) Perfusion with 2×10^{-6} M AChI for 11 min between first and second sets of recordings. Slight depolarization, further diminution in force (33% from control).

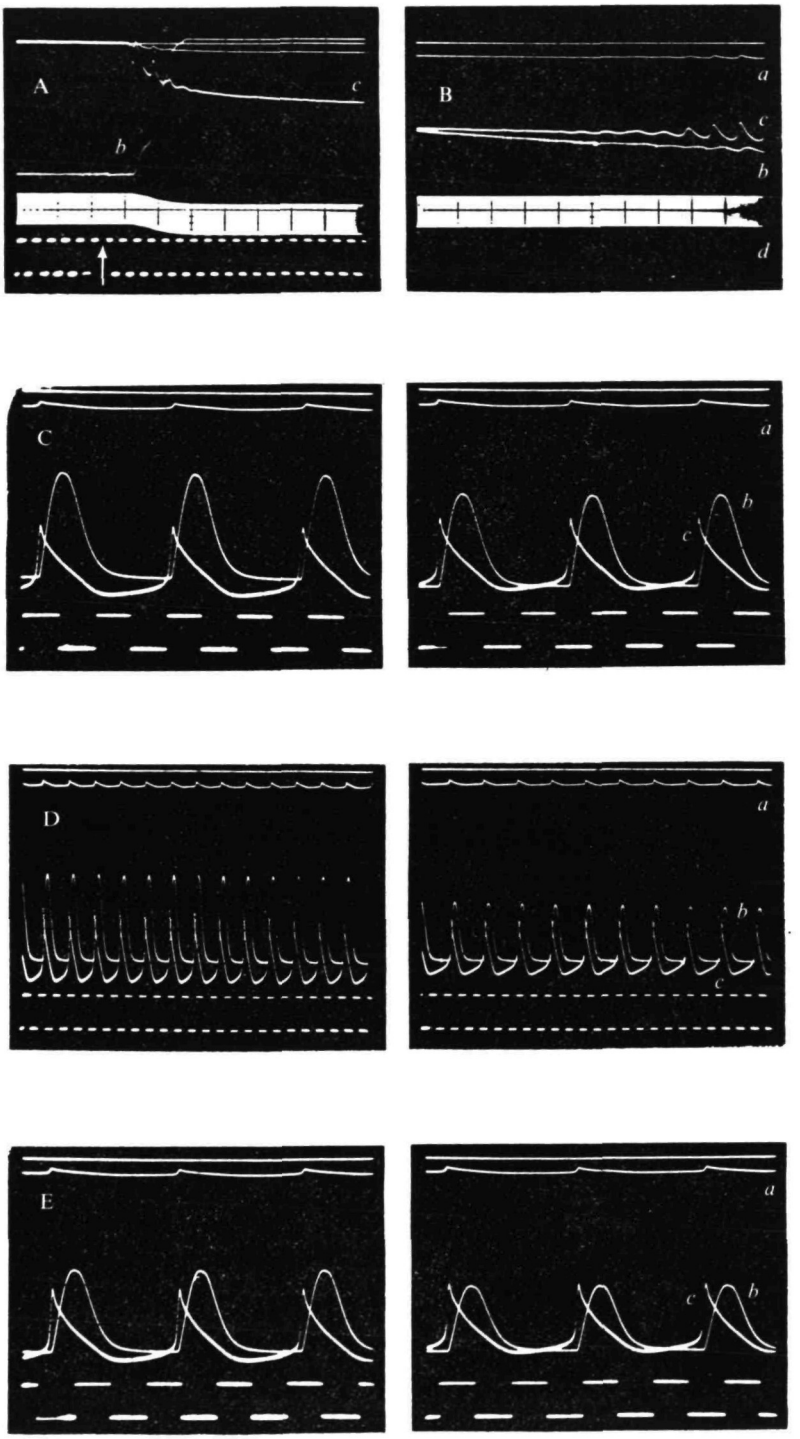

Fig. 1. For legend see facing page.

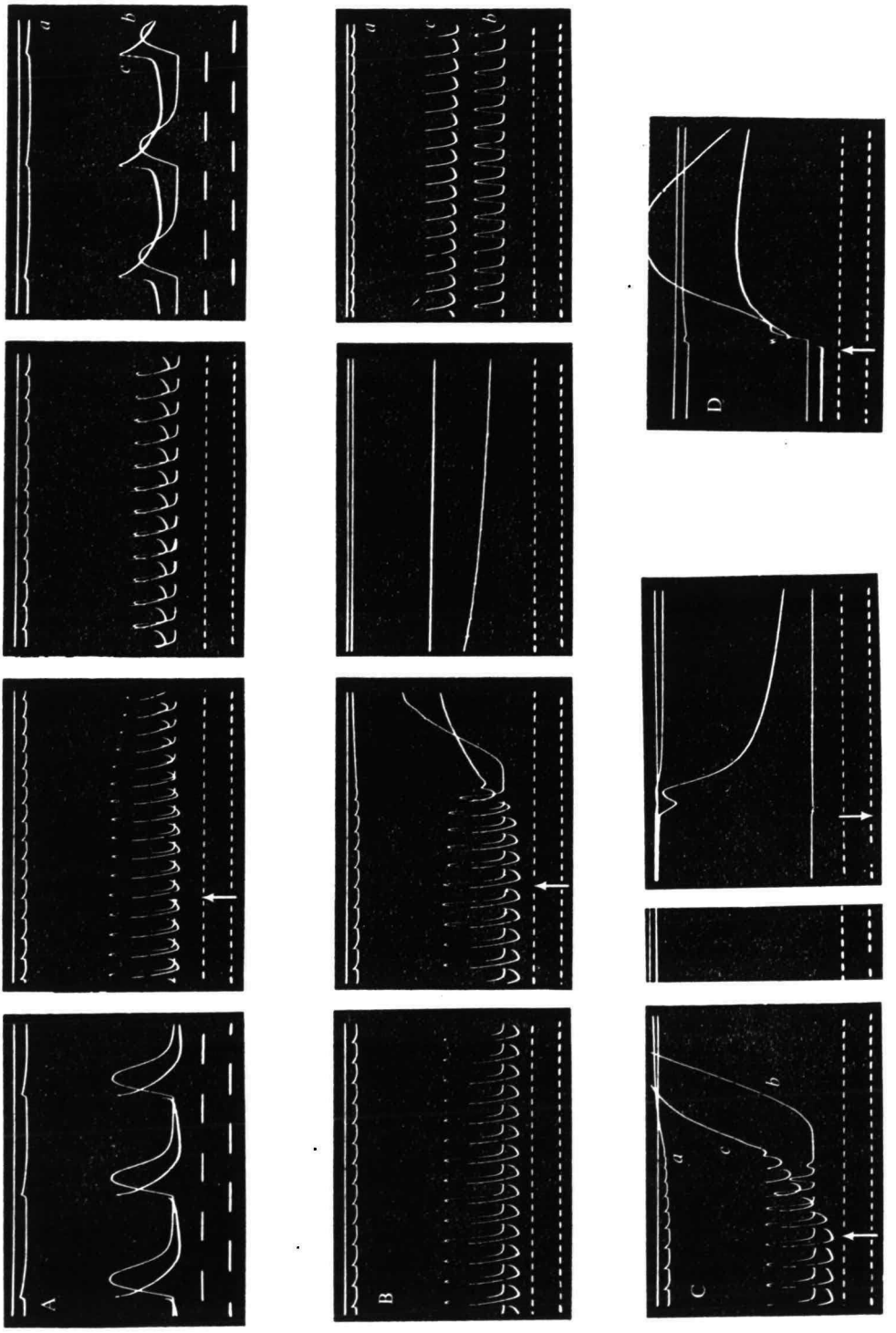

Fig. 2. Calibrations as in Fig. 1. (A) Superfusion with 2×10^{-5} M AChI at the arrow. Last set of recordings after 20 min of superfusion. Depolarization (nominal 3·3 mV) and 60% reduction in force from control. Slight negative chronotropic effects. (B) Superfusion with 2×10^{-4} M AChI at the arrow, resting potential 28 mV. Marked depolarization and contracture, but after 6 min of superfusion, beating with reduced force at a depolarized level (last set of recordings). (C) Superfusion with 2×10^{-3} M AChI at the first arrow, resting potential, 24 mV. Complete depolarization accompanied by contracture. (D) Superfusion with 10^{-3} M AChI. Depolarization accompanied by contracture.

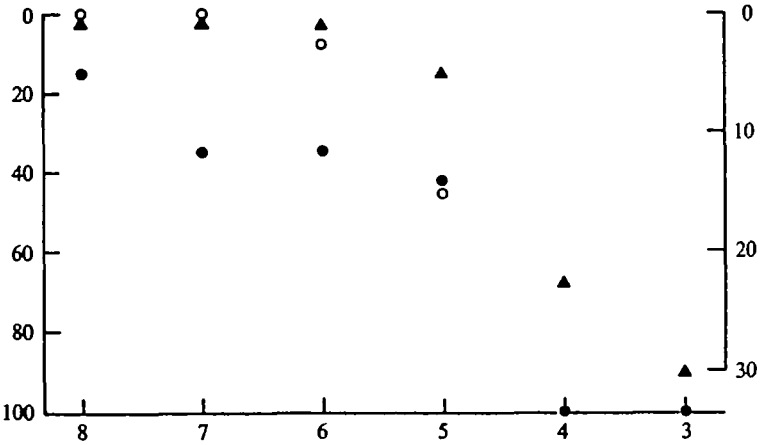

Fig. 3. Left ordinate: percentage decrease in contractile force (●) and in amplitude of action potentials (○). Right ordinate: resting potential in mV (triangles). Abscissa: negative logarithm of ACh iodide concentration.

control value in sea water without ACh at the beginning of the experiment. At 2×10^{-3} M (Fig. 2C) superfusion with ACh depolarized completely (even reversed past the end depolarized with KCl) and induced a powerful contracture, at 21 mV depolarization, that lasted until the ACh was washed off. Subsequent superfusion with 10^{-3} M ACh (Fig. 2D) depolarized by 14 mV and induced a contracture which subsequently relaxed.

Acetylcholine was depolarizing at all concentrations from 10^{-8} to 10^{-3} M (Fig. 3). The negatively inotropic effects of ACh are therefore not hyperpolarizing, at least at the level of the overall effect on the ventricular myocardium. However, a negative effect on force was already apparent at 10^{-8} M and at 10^{-7} M, which are concentrations of ACh below the threshold for an effect on amplitude of action potentials. At high concentrations (10^{-4} M, 10^{-3} M) depolarization was accompanied by arrest of the heart-beat in contracture, but at intermediate concentrations diminution of force of beats accompanied diminution in amplitude of action potentials. Therefore the negatively inotropic effect at high concentrations may be attributed to depolarization contracture, and the negatively inotropic effect at intermediate concentrations may be attributed to diminished amplitude of action potentials. The negatively inotropic effect at low concentrations still requires explanation.

INTERACTION OF 5HT AND ACH

Once the plateau on the action potential, induced by 10^{-3} M 5HT, had become established (Fig. 4A, B), the effect of 2×10^{-7} M ACh was to shorten the plateau (Fig. 4C–F). Force was reduced in proportion to the shortening of the plateau.

In 10^{-3} M 5-HT (Fig. 4B) time to half-repolarization was 0·6 sec. Force reached 7·5 g (Fig. 4A). The addition of 2×10^{-7} M ACh to the perfusing medium (Fig. 4C) led to a steady shortening of the AP and diminution of force. After 7 min, time to half-repolarization of the action potential was 0·5 sec (Fig. 4F). The 'plateau' portion of the repolarizing phase was noticeably shortened. Force fell to 4·2 g (Fig. 4E).

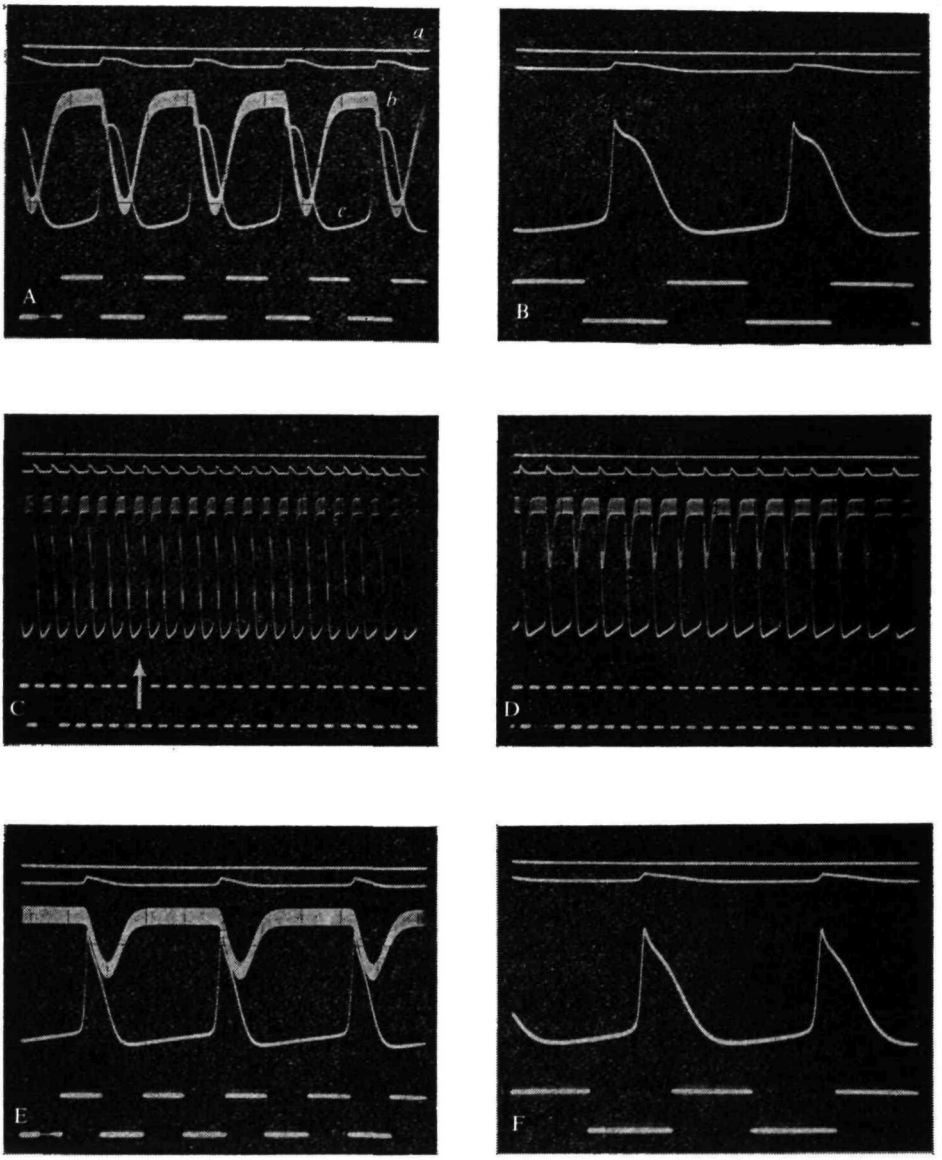

Fig. 4. Interaction of ACh and 5-HT on the duration of the plateau of the cardiac action potential and consequent effect on force of contraction. (A) Effect of 10^{-8} M 5-HT after 3 min of superfusion. (B) After 11 min, to show form of the cardiac action potential. (C) Beating in 10^{-8} 5-HT, 2×10^{-7} M ACh added to the superfusion at the arrow. (D) After 3 min, force is reduced. (E) After 5 min, for comparison with A. (F) After 7 min, for comparison of form of AP with B. *a*, Gap potential monitor; calibration, 50 mV; *b*, force; calibration, 2·5 g, increase in force-downward deflexion; *c*, AP; calibration 10 mV.

A low concentration of acetylcholine has an opposite effect to 5-HT, on the duration of the plateau of the cardiac action potential. The shortened duration of the plateau is accompanied by a decrease in force, as one would predict if the duration of depolarization directly governs force by determining the availability of free calcium ion for excitation-contraction coupling. This observation depends on the use of some means

lengthening the plateau, such as perfusion with 5-HT, since the effect of ACh on plateau length is not immediately apparent in hearts beating spontaneously with a short duration of the plateau phase of the cardiac action potential.

DISCUSSION

The results suggest that ACh and 5-HT may interact on force by affecting the length of the cardiac action potential, thus affecting the duration of the period during which excitation–contraction coupling can occur. Previous experiments on the ventricles of *Dolabella auricula* (Nomura, 1965), of *Busycon canaliculatum* (Kuwasawa & Hill, 1973) and of *Aplysia dactylomela* (Hill & Yantorno, personal communication) suggest that E–C coupling is effected by entry of Ca^{2+} into the myoplasm, probably from the extracellular compartment. Since 5-HT increases the duration of the cardiac action potential, it is probable that the increase in force results from an increased time for influx of Ca^{2+}. ACh reduces the force of spontaneously beating ventricles, but low concentrations did not affect the amplitude of spontaneous action potentials. However, after extending the duration of action potentials with 5-HT it could be seen clearly that ACh would then shorten the plateau of cardiac action potentials. Again, it is probable that the accompanying decrease in force results from a shortened time for influx of Ca^{2+}.

The threshold for ACh on the isolated ventricle of *Dolabella auricularia* (around 2×10^{-8} M) corresponds very closely to the threshold concentration, for inotropic effects, of externally applied ACh on the isolated collapsed ventricle of *Aplysia dactylomela* (Hill, 1964). The threshold was very much lower (between 10^{-13} and 10^{-12} M) when acetylcholine was applied by perfusion through the lumen of the isolated ventricle of *Aplysia fasciata* (Hill, 1964). However, superfusion of the *Dolabella* ventricle in one end-compartment of the sucrose gap apparatus does in fact amount to external application. At 2×10^{-4} M, ACh depolarized the isolated *Dolabella* ventricle and a contracture accompanied the depolarization. At the same concentration level a similar contracture was recorded mechanically from the isolated ventricle of *Aplysia dactylomela* (Hill, 1964).

The triphasic concentration–action curve for ACh, which was found for the hearts of the prosobranch gastropods *Busycon canaliculatum* and *Strombus gigas* (Hill, 1958; Hill & Thibault, 1968), is apparently reduced to a biphasic curve for the heart of the opistobranch gastropods *Aplysia dactylomela* (Hill, 1958), *Aplysia fasciata* (Hill, 1964) and *Dolabella auricularia*. That is, ACh has a negatively inotropic effect at low concentrations and a positively tonotropic (increase in tonus) effect at high concentrations. The present studies show that ACh depolarizes at all concentrations, with a direct relation between concentration and depolarization in both the low and high ranges. However, at low concentrations the slight, long depolarization is accompanied by a profound loss of force. These low concentrations also correspond to the range at which ACh has been found to increase the rate of relaxation in contractions of gastropod venticles (Hill, 1967). The shortening of the plateau of cardiac action potentials by low concentrations of ACh in the presence of 5-HT might be expected to diminish the concentration of activator Ca^{2+} released into the myoplasm in each contraction. The negatively inotropic effect of low concentrations

of ACh may therefore be related not to the slight depolarization, but to an effect on activator (Ca^{2+}), mediated through the effect on plateau duration.

The contracture caused by depolarization with high concentrations of ACh agrees with the relationship between depolarization and tension obtained for the radula protractor (Hill *et al.* 1970), and is thus not a particular property of gastropod cardiac muscle.

The mixed inhibitory and excitatory effects at intermediate concentrations (e.g. 5×10^{-6} M) may then be explained as an interaction. At these concentrations the negatively inotropic effect of ACh on a beating ventricle (Hill, 1964) may be due to an indirect effect on E–C coupling, whereas the positively inotropic and chronotropic effects (on rate), which may be superimposed on an increase in tone of a quiescent heart (Hill, 1964), may be due to excitation by depolarization (Kuwasawa & Matsui, 1970).

The triphasic curve in prosobranch ventricles is also consistent with a depolarizing effect throughout, since depolarization may explain both the positively chronotropic effect at very low concentrations and the contracture at high concentrations. The negatively inotropic effect at intermediate concentrations is consistent with the shortening of action potential plateaus.

Comparison with bivalve hearts

Wilkens & Greenberg (1973) report that ACh only excited the heart of *Modiolus*, although they describe an effect of 10^{-4} M ACh very similar to that shown for *Dolabella* (Fig. 2B), except that the increase in rate that accompanied resumed beating was somewhat greater for *Modiolus*.

Ostrea laperousei – now identified as *Crassostrea gigas* (M. J. Greenberg, personal communication) – has a cardiac action potential with typical spike and plateau configuration (Irisawa, Kobayashi & Matsubayashi, 1961*a*). Irisawa *et al.* found that ACh had no depolarizing effect, but that the plateau was affected as in *Dolabella* ventricle: 10^{-4} ACh reduced the action potentials to a slight oscillation of PD, the spike recovering before the plateau after treatment. Force was markedly reduced when the plateau was abolished by a current pulse (Irisawa *et al.* 1961*b*). Force is therefore probably dependent upon duration of the AP in both bivalve and gastropod ventricle.

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