J. Physiol. (1954) 125, 208–214

THE ACTION OF ACETYLCHOLINE ON THE HEARTS OF LAMELLIBRANCH MOLLUSCS

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(*Received* 11 *February* 1954)

The action of the acetylcholine group of compounds on the hearts of bivalve molluscs (lamellibranchs) has been studied in very few species. The published results may be classified and summarized as follows:

ACh applied to isolated preparations of the ventricles of lamellibranchs either

(1) decreases frequency and amplitude

(*a*) in high concentrations: oyster (*Gryphaea angulata*?) at 10^{-5}, with diastolic arrest following at $2 \times 10^{-5} - 2 \times 10^{-4}$ (Jullien, 1935),

(*b*) in low concentrations: clam (*Venus mercenaria*) at 5×10^{-12}, with diastolic arrest following at *c.* $2{\cdot}5 \times 10^{-10}$ (Wait, 1943),

or (2) inhibits beat and produces a rise in tone or marked 'contracture': mussel (*Mytilus gallo-provincialis*) at $10^{-5} - 10^{-3}$ (Jullien & Vincent, 1938).

These results do not appear to show any overall behavioural pattern and no attempt has been made, as far as the writer is aware, to draw them together. Jullien & Vincent (1938) themselves regarded the reaction of *Mytilus* as exceptional with respect to the behaviour of mollusc preparations in general. Bacq (1947) and Fredericq (1947) have made surveys which include aspects of the physiology and pharmacology of molluscan heart tissues without appearing able to do more than draw attention to the lack of coherent knowledge concerning the Mollusca. Welsh and co-workers in America have followed up the early observations of Prosser on *Venus* (1940), but chiefly towards an examination of the fundamental action of ACh in general and the importance of various aspects of the ACh molecule. (See Welsh & Taub, 1950, for references.) The present work was undertaken to examine the effect of ACh applied to preparations of isolated ventricles from a number of different species of lamellibranchs. The results indicate that the gaps in the recorded observations (above) are

due to the restricted range of species so far used by other workers and that a continuous series of effects can be demonstrated ranging from very sensitive to much less sensitive species. The apparently anomalous situation obtaining in *Mytilus* (Jullien & Vincent, 1938) is now found in other genera including some in which the more usual inhibitory effects occur at low concentrations.

METHODS

Acetylcholine chloride (B.D.H.) was used in all experiments; it was made up weekly in a concentration of 10^{-3} (w/v) ACh in 5% (w/v) NaH_2PO_4, stored in a refrigerator and diluted as required to convenient concentrations in sea water so that the actual amount of solution added to the bath was no greater than 2·5 ml. Preparations were tested in ACh concentrations of 3×10^{-12}–10^{-4} and, where they appeared critical, at intermediate values, as $3\times$, $5\times$, $6\times$, 10^{-n}. The drug was added by means of a pipette to the beaker, a stream of air bubbles ensuring rapid mixing; washing was effected by replacing the medium as in the earlier experiments (Pilgrim, 1953); 10–30 min were usually sufficient to allow complete recovery. The lever tension varied from *c.* 50 mg to *c.* 200 mg according to the size of the preparation. Temperature was not controlled; it varied from 9·6 to 18·8° C over the whole investigation, but during any one experiment was constant to within ±0·5° C.

The animals used all belong to the order Eulamellibranchia except *Mytilus* (Filibranchia); no Protobranchia or Septibranchia were available. They comprised: *Dosinia anus* (Philippi), a large species (shell-length of average specimens 7 cm), normally found in sublittoral sandy situations around the New Zealand coasts; the specimens used were limited to those collected in the littoral zone, probably occurring there temporarily after storm disturbance to the sea bed. Without dredging facilities a constant supply is not assured.

Amphidesma forsterianum Finlay (5·5 cm), extremely abundant in sandy littoral beaches near Christchurch.

A. ventricosum (Gray) (7 cm), the *Toheroa* of commerce, common only in the North Island, is not found near Christchurch.

Protothaca crassicosta (Deshayes) (4 cm), common beneath rocks in the littoral zone.

Chione stutchburyi (Gray) (3 cm), a very abundant cockle in estuaries throughout New Zealand.

Ostrea hefferdi Finlay (6 cm), an oyster common on rocks in the littoral zone.

Mytilus canaliculus Martyn (10 cm), a mussel extremely abundant on rocks in the littoral zone throughout New Zealand.

Hyridella menziesi (Gray) (8 cm), common in quiet fresh waters connected to river systems in the South Island.

The preparations were set up as described by Pilgrim (1953), except that it was found more convenient for small animals to tie the threads at the auriculo-ventricular junctions as described by Wait (1943), and this procedure was adhered to for all species. Sea water (salinity 33·0—34·5‰) was the bathing medium used for all marine species and 5% sea water for *Hyridella*, the dilution being made with M/400-$NaHCO_3$. It has been shown (Pilgrim, 1953) that these media are suitable for lamellibranch preparations from marine (or estuarine) and fresh-water habitats respectively. The preparations were usually inert when first set up, but a regular rhythm was attained in 2 or 3 hr in most cases; observations were made over the first 10 min after adding the drug, the reactions mostly becoming evident within half a minute. A total of 469 experiments was made on ninety-six separate preparations.

RESULTS

The results of this investigation are illustrated in Figs. 1–3, the preparations being arranged in order of decreasing sensitivity to ACh. The extreme limits are incorporated in the figures, but individual preparations did not show so

much overlap of reactions as do the pooled results: data from the literature have been added for comparison. ACh concentrations are plotted logarithmically.

Amplitude decrease and diastolic arrest (Fig. 1)

These occurred over a fairly wide range of drug concentrations, but there is nevertheless a distinct trend in the reactions of the species from very sensitive, resembling *Venus* (e.g. *Dosinia* and *Amphidesma*) to relatively

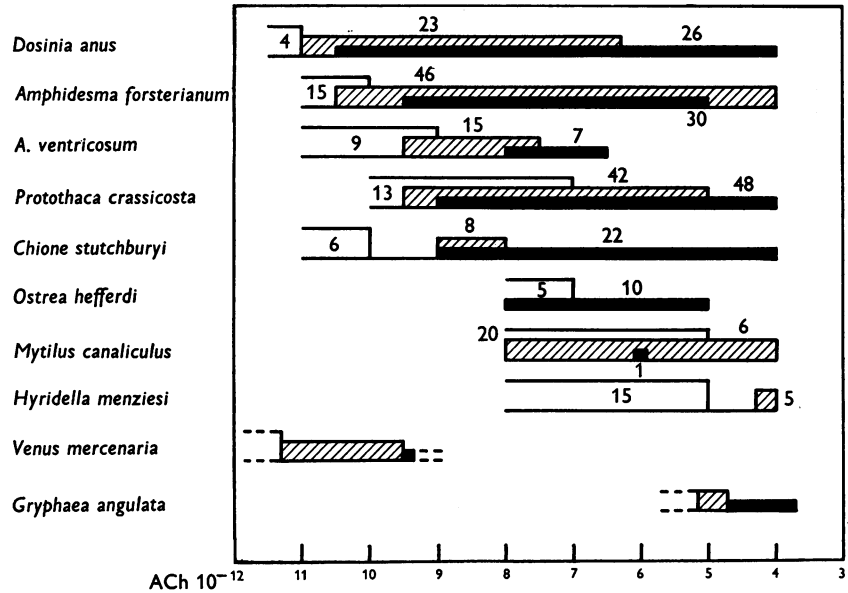

Fig. 1. Results of experiments showing range of drug concentrations in which amplitude was reduced or diastolic arrest occurred. In Figs. 1 and 2, results from this paper are arranged in order of decreasing sensitivity to ACh and data from the literature are added below. Open areas mean no effect; shaded areas, reduced activity; black areas, diastolic arrest. In all figures ACh concentration is plotted logarithmically, and the numbers refer to the number of experiments on which the results are based.

insensitive, resembling *Gryphaea* (e.g. *Ostrea* and *Mytilus*). Diastolic arrest was found only once in *Mytilus* and not at all in *Hyridella*; this will be discussed later. The apparent absence of amplitude decrease in *Ostrea* (Fig. 1) may be due to its not being detected owing to the smallness of the beat, even when magnified by the lever.

Frequency decrease (Fig. 2)

In most preparations frequency was reduced, by 10–15%, along with amplitude, so that the graphed results (Figs. 1 and 2) are similar for species on which ACh has an inhibiting action. It is evident, however, that amplitude is usually affected at weaker concentrations of the drug where frequency may

be unchanged. This is in agreement with Prosser (1940) who found that ACh had primarily a negative inotropic effect on *Venus*.

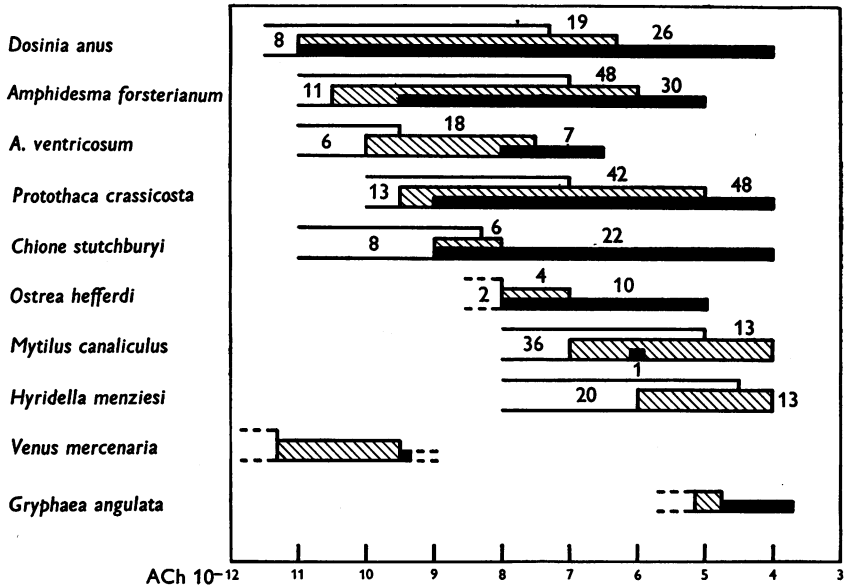

Fig. 2. Results of experiments showing range of drug concentrations in which frequency was reduced or diastolic arrest occurred. (Same experiments as in Fig. 1.)

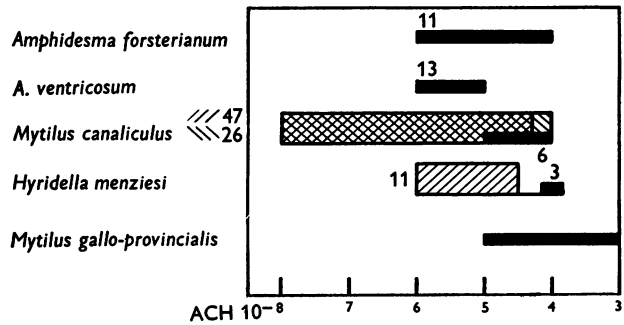

Fig. 3. Results of experiments showing range of drug concentrations in which amplitude and frequency were increased or systolic arrest occurred. Shaded areas represent amplitude (////) and frequency (\\\\); black areas mean systolic arrest.

Amplitude and frequency increase and systolic arrest (Fig. 3)

The first two of these phenomena do not seem to have been reported before; all three were found as sustained features in many preparations of *Mytilus canaliculus*, while in *Hyridella* high concentrations of ACh often led to amplitude increase without a lasting change in frequency, and, in ACh at 10^{-4}, to systolic arrest. The occurrence of systolic arrest in *Amphidesma forsterianum*

(eleven examples) and *A. ventricosum* (thirteen examples) is here regarded as of similar significance, but it is to be noted that there was never any instance of associated lasting increase in amplitude or frequency: in both species it was commonly found that ACh at 10^{-7} produced diastolic arrest; the same preparation in ACh at 10^{-6} showed distinct though brief increase in amplitude and frequency before going into a sustained 'contracture', and in ACh at 10^{-5} went immediately into contracture. *Hyridella* often showed the latter two reactions at drug concentrations 10^{-5} and 10^{-4} respectively.

DISCUSSION

The above experiments show that there is some mechanism, intimately linked with the rhythmically contractile system of the lamellibranch ventricle, which is capable of being influenced by ACh, and that the concentration of the drug which is effective varies between species; preparations from some species commonly experience a reduction in activity at concentrations which do not affect other species (e.g. *Dosinia* and *Hyridella* in ACh at 10^{-10}—10^{-7}). The gradation of response links together the earlier inconsistent reports of other workers, and may well be due to quantitative differences in the histological composition of the material. The evidence from the present work is not sufficient to permit postulating a cholinergic transmission at the ventricle of these animals, as Prosser (1940) was able to do for *Venus*, but the possibility is by no means unlikely and the varying sensitivities may be due to varying numbers of reactive elements in the tissue. Our knowledge of the histology of the lamellibranch heart is particularly deficient and controversial (Prosser, 1942) and will repay investigation with modern methods. The rediscovery of the *Mytilus*-type of reaction to high ACh concentrations is particularly significant in that it is also found in other species, including some in which the depressant effect of ACh at low concentrations brings them into line with the remaining species. The results showing acceleration and enhancement of the beat upset the simple classification of invertebrate heart reactions given by Prosser (1942). It is here suggested that the systolic rise in tone, with accompanying transient or lasting increase in amplitude and frequency, and ultimately systolic arrest, represent an action of the drug primarily on the contractile elements themselves (cf. the effect of high concentrations of ACh on mammalian voluntary muscle), though the fact that frequency does change may mean that the pacemaker is also affected. It is interesting in this connexion that a few preparations of *Amphidesma forsterianum* and *Hyridella* which failed to beat, nevertheless showed the characteristic strong rise in tone in ACh at 10^{-4}, indicating that their inertness was due to pacemaker failure and that their contractile mechanism was still functional. Prosser (1940) found a contraction in response to mechanical or electrical stimulation of ACh-inhibited *Venus* heart.

On the basis of the above postulates, the following is offered as an explanation of the behaviour of those preparations which are both depressed at low concentrations and stimulated, finally to contracture, at high concentrations of ACh: the low concentration tends to inhibit the pacemaker activity or some (neural?) element in the chain leading to contraction of the muscle but the drug is not at this concentration capable of itself evoking contraction; at high concentrations the drug acts directly on the muscle, the pacemaker being quite inhibited, allowing a steady sustained contraction. In *Mytilus* and *Hyridella* the 'contracture' reaction must be so pronounced as to obliterate diastolic arrest. The initial rapid beats of *Amphidesma* prior to contracture may be explained as due to the drug not reaching the reactive elements in full strength immediately. These three stages may well correspond to Prosser's pacemaker, conduction and contraction mechanisms, and it may not be necessary to postulate nervous elements in the case of isolated preparations.

The very sensitive species, *Dosinia* and *Amphidesma* are comparable to *Venus* in their ACh reaction, and should be equally suitable for bioassay of this substance. Further work is being pursued with the ACh-group and other drugs on *Amphidesma forsterianum* and *Mytilus canaliculus*, which are chosen for their availability, hardiness of preparation and, to ACh at least, contrasting behaviour.

SUMMARY

1. Isolated ventricle-strip preparations from eight species of New Zealand lamellibranchs were submitted to the action of acetylcholine in concentrations of 3×10^{-12} to 10^{-4}.

2. Some species were inhibited, finally to diastolic arrest at high concentrations; *Mytilus* and *Hyridella* showed increased activity but went into a contracture at high concentrations; others gave either reaction at appropriate concentrations and connect the species into a graded series. The more sensitive species are suggested as suitable for bioassay of ACh.

3. The results are considered along with those of other workers, and it is shown that the gaps in the recorded observations are now bridged.

4. An hypothesis is offered to explain the results described.

I wish to thank Prof. E. Percival, F.R.S.N.Z., for providing facilities and for his encouragement in this work; the experiments were carried out at the suggestion of Dr W. Feldberg, F.R.S., to whom I am greatly indebted for advice and criticism. I acknowledge the receipt of a Research Grant from the University of New Zealand which partly defrayed the expenses incurred.

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