EFFECT OF SOME MYOTROPIC SUBSTANCES ON MOLLUSC HEARTS

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Summary

The hearts from a number of estuarine molluscs typical of eastern Australian waters have been examined for their response to acetylcholine, adrenaline, noradrenaline, and serotonin. Under the conditions of these experiments *Anadara trapezia* showed the highest sensitivity to acetylcholine, being depressed by 0·001 μg/ml. Adrenaline in concentrations of 0·005 μg/ml produced a decrease in the amplitude of contractions of *Regozara flava* heart which also responded to 0·01 μg/ml noradrenaline. Hearts from other species did not respond to catecholamines. *Fragum unedo* and *Regozara flava* heart showed greatest sensitivity to serotonin which increased amplitude of contraction. *Pinna menkei* heart was unresponsive to myotropic substances under the conditions used. The factors affecting sensitivity of response to myotropic substances are discussed.

I. Introduction

The phylum Mollusca has been shown to contain a number of species which display remarkable sensitivity to various vertebrate transmitter substances. Thus the cephalopod heart responds to concentrations of adrenaline and noradrenaline which are of the same order as those which produce a response in mammalian tissue (for references see Fänge 1962). Gaddum and Paasonen (1955) were able to detect 1×10^{-4} μg/ml serotonin using the isolated heart of *Spisula solida* (Dillwyn) and Prosser (1940) showed that *Mercenaria* (= *Venus*) *mercenaria* (L.) heart responded to 1×10^{-6} μg/ml acetylcholine.

A survey of molluscs for suitability as test objects for the assay of acetylcholine was carried out at Sydney by Ladd and Thorburn (1955) who found the tapestry cockle, *Tapes watlingi* (Iredale) (= *T. turgida* of Lamarck) was similar to *V. mercenaria* in sensitivity and specificity for acetylcholine. The possibility of extending the use of molluscs found in Australian waters for the assay of myotropic substances, and in particular of adrenaline and noradrenaline, led to a survey being undertaken. The present paper reports some typical findings with respect to the response of both fresh-water and marine lamellibranchs.

II. Materials and Methods

(*a*) *Specimens*

The salt-water specimens were collected at low tide from sand or mud banks at various sites in Moreton Bay. After collecting, the molluscs were kept in aerated

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sea water until required for testing. The fresh-water specimens were obtained from a fresh-water stream near Brisbane and had been kept alive in the laboratory for a number of months before use.

(*b*) *Apparatus*

The apparatus consisted of a 10 ml glass organ-bath and a glass aerating tube. The tissue was attached by a thread at one end to the aerating tube and a thread from the other end of the tissue passed to an isotonic ink-writing gimbal lever, the movements of which were recorded on a kymograph drum. The lever system provided a basic load of 100 mg on the tissue.

(*c*) *Media and Chemicals*

Determinations of sodium, potassium, magnesium, and calcium were made on sea water and the extra-pallial fluids of some lamellibranchs by means of atomic absorption spectroscopy. Hearts from marine specimens were suspended in undiluted aerated sea water. The heart from the fresh-water specimen was suspended in a dilution of one part sea water to nine parts distilled water. The adrenaline and noradrenaline solutions were prepared as described in the British Pharmacopoeia (1963) at strengths of 1 mg/ml. The serotonin was prepared in a 0·1 mg/ml solution as the creatinine phosphate of 5-hydroxytryptamine. Acetylcholine was prepared as a 1 mg/ml solution in 5% sodium dihydrogen phosphate as described by Wait (1943) and in all cases the substance was diluted with the appropriate medium immediately before use.

(*d*) *Collection of Extra-pallial Fluid*

The extra-pallial fluid was removed from the marine specimens immediately on collection. In all specimens the fluid was withdrawn from the extra-pallial sinus with a syringe fitted with a large hypodermic needle. The valves were opened only sufficiently to allow the entry of the needle.

(*e*) *Preparation of Specimen*

In all cases one of the valves was removed by inserting a sharp implement between the mantle and lip of the shell and carefully severing the adductor muscles. The pericardium was removed and fine threads were attached at the atrio-ventricular junctions. The ventricle was then carefully removed to avoid tearing and attached to the apparatus.

It was generally found that the hearts from most species did not beat immediately of their own accord. Specimens of *Anadara* collected in early spring showed spontaneous activity but those collected at other times of the year remained refractory for long periods. Efforts were made to initiate beating by adding small quantities of ethanol, fructose, glucose, or serotonin to the media. Ethanol, glucose, and fructose were ineffective. In most cases rhythmic activity was initiated by serotonin which was then washed from the preparation. The heart usually continued to beat regularly after this treatment and could be used for the determination of responses to other drugs.

III. Results

(*a*) *Investigation of the Ionic Content of Extra-pallial Fluid*

The extra-pallial fluids from representative species of molluscs were assayed for sodium, potassium, calcium, and magnesium to see whether specific media would be needed to maintain their ventricular activity. Published values for these cations in sea water show that they are remarkably constant. The results of the cation determinations on extra-pallial fluids and sea water are shown in Table 1.

The cation concentrations of the extra-pallial fluid from marine molluscs was similar to that of sea water except that K^+ tended to be higher and Ca^{2+} lower. This is contrary to the findings of Hayes and Pelluet (1947) who found the K^+ content to be much the same as in sea water.

Table 1

Ionic concentrations in sea water and extra-pallial fluid

Specimen	Sodium Concn. (g/l)	Potassium Concn. (g/l)	Calcium Concn. (g/l)	Magnesium Concn. (g/l)
Sea water	10·9	0·39	0·43	1·37
Anadara trapezia	10·6	0·49	0·33	1·29
Cucumerunio novaehollandiae	0·75	0·03	0·12	0·22
Regozara flava	10·9	0·48	0·36	1·33
Fragum unedo	10·9	0·42	0·33	1·21
Antigona lamellaris	10·9	0·46	0·36	1·23
Tapes watlingi	11·0	0·50	0·36	1·27

Dugal (1939) has shown that the calcium content of mollusc tissue fluids varies, increasing in proportion to the duration of closure of the valves. Since this has no apparent effect on mollusc tissues, they must be resistant to moderate variations in cation concentration. Accordingly, hearts from marine animals were suspended in sea water which, as shown in Table 1, has values approximating to the values of the various extra-pallial fluids. In the case of *Cucumerunio*, which has its habitat in fresh-water streams with very low ionic content, a 1 in 10 dilution of sea water approximates to the values obtained by the analysis of extra-pallial fluid.

(*b*) *Responses of the Hearts of Different Species of Molluscs to Various Myotropic Substances*

(i) *Family Arcidae*

Anadara trapezia Deshayes heart could only be made to beat in early spring, even serotonin being without effect at other times. In weakly beating hearts serotonin had positive tonotropic, chronotropic, and inotropic effects. Adrenaline and noradrenaline were without effect on the heart either alone or in the presence of serotonin, even in concentrations as high as 1 μg/ml. Acetylcholine exerted a negative chronotropic effect and in high doses (0·1 μg/ml), a negative inotropic effect, and a positive tonotropic effect.

(ii) *Family Pinnidae*

Pinna menkei Reeve heart beat spontaneously and the rhythm became quite regular with increasing load. Neither adrenaline at 5 μg/ml nor noradrenaline at 1 μg/ml produced any observable effect. Concentrations up to 0·1 μg/ml acetylcholine were also without effect. A very slight increase in tone and amplitude was produced by 0·1 μg/ml serotonin.

(iii) *Family Mutelidae*

The heart from one specimen of *Cucumerunio novaehollandiae* Gray commenced to beat after stimulation with serotonin (0·1 μg/ml) but quickly stopped again. The heart was again started with serotonin 2 hr later and continued to beat strongly but rather slowly. It failed to respond to 0·5 μg/ml adrenaline, 1·0 μg/ml noradrenaline, 0·05 μg/ml acetylcholine, 20 μg/ml K^+ (as KCl), or 5 μg/ml Mg^{2+} (as $MgSO_4$). The effect of the serotonin wore off after 1 hr but a further dose re-established the heart action. Increases in serotonin concentration above 0·1 μg/ml were without additional effect.

(iv) *Family Cardiidae*

Cardium setosum Redfield heart was active and distended *in situ* but did not contract spontaneously when removed from the animal. Activity was initiated and maintained by the addition of 0·1 μg/ml serotonin. A feature of *C. setosum* was the delay of approximately 1 min before any response to the myotropic substances was exhibited. Adrenaline at 1·0 μg/ml stopped the heart in diastole. A decrease of 20% in rate was produced by 0·3 μg/ml acetylcholine without any change in tone or amplitude.

Adrenaline produced a decrease in the amplitude and frequency of contraction of *Regozara flava* L. heart. In fresh preparations adrenaline at concentrations of 0·005, 0·01, and 0·02 μg/ml produced a decrease in amplitude of *c.* 6%, 16%, and 25%, respectively. With older preparations (18–22 hr after setting up) the response was only half that of the fresh preparation. Gentle stretching of the heart muscle by a temporary increase in tension restored the sensitivity of the preparation to adrenaline so that 0·02 μg/ml adrenaline caused a 40% decrease in amplitude. The sensitivity was not altered during the stretching period which lasted for 10 min. In the presence of serotonin the effect of adrenaline on amplitude was transient and significantly less than normal. The decrease in frequency of contractions produced by adrenaline was antagonized by pretreatment with serotonin. Adrenaline lowered the tone. Incubation at room temperature for 2 hr in the presence of 0·05 μg/ml adrenaline produced a refractory preparation. Noradrenaline did not produce as great an effect on frequency, amplitude, or tone as adrenaline and did not influence the response to adrenaline itself. Acetylcholine produced a decrease in amplitude and frequency and a slight decrease in tone.

In *Fragum unedo* L. heart, doses of up to 0·1 μg/ml serotonin caused transitory minor increases in frequency and increases of muscle tone. When the heart was beating against a basic load of 100 mg, 0·1 μg/ml serotonin increased the amplitude by 300%. Loading the heart to 500 mg made it more sensitive to serotonin so that 0·01 μg/ml, which was without apparent effect at the lower load, now produced a

90% increase in amplitude. Acetylcholine produced a disruption of heart rhythm, a decrease in resting tone, and a decrease in both force and frequency of contraction. Both noradrenaline and adrenaline in doses of up to 0·1 μg/ml, either alone or in the presence of serotonin, had no effect.

(v) *Family Veneridae*

Both *Periglypta* species tested (*P. puerpera* L. and *P. resticulata* Sowerby) have extremely thin-walled fragile hearts and were unsatisfactory on this account. *P. puerpera* did not commence beating and *P. resticulata* beat weakly and was only slightly stimulated by serotonin which produced an increase in both amplitude and frequency.

TABLE 2

SENSITIVITY OF MOLLUSC HEARTS TO MYOTROPIC SUBSTANCES

"—" signifies no tonotropic, chronotropic, or inotropic effect to myotropic substance up to the concentration stated. "+" signifies a response at the concentration stated

Species	Adrenaline		Noradrenaline		Serotonin		Acetylcholine	
	Concn. (μg/ml)	Response	Concn. (μg/ml)	Response	Concn. (μg/ml)	Response	Concn. (μg/ml)	Response
Anadara trapezia	1·0	—	1·0	—	0·1	+	0·001	+
Pinna menkei	5·0	—	1·0	—	0·1	—	0·1	—
Cucumerunio sp.	0·5	—	1·0	—	0·1	+		
*Cardium setosum**	0·2	+			0·05	+	0·3	+
Regozara flava	0·005	+	0·01	+	0·01	+	0·1	+
Fragum unedo	0·1	—	0·02	—	0·01	+	0·02	+
Periglypta resticulata					0·1	+		
Antigona lamellaris	1·0	—	1·0	—	0·1	+	0·1	+

* In presence of 0·1 μg/ml serotonin.

Antigona lamellaris Schumacher ventricles commenced spontaneous beating at a rate of approximately 6 beats/minute as soon as they were set up. Neither adrenaline nor noradrenaline had any observable effect on the heart whether it was under light or moderate load. Acetylcholine lowered the frequency of beating by 20% and decreased the amplitude slightly. Tonotropic effects were not significant. Serotonin at a concentration of 0·1 μg/ml increased amplitude by 25–30% and frequency by 65–75%. With light loading the resting tension was slightly reduced but with moderate loading serotonin had a positive tonotropic effect.

(c) *Sensitivity of Mollusc Hearts to Various Myotropic Substances*

Table 2 shows the sensitivity of various mollusc hearts to four myotropic substances. Of the specimens examined *Regozara flava* was the only one that responded to both adrenaline and noradrenaline, higher concentrations of noradrenaline being required to elicit the response. All hearts responded to serotonin and only *Cucumerunio* sp. and *Pinna menkei* showed no response to acetylcholine. In the case of *Cardium setosum* a response to acetylcholine was elicted by 0·3 μg/ml, a greater

concentration than that used for any other species. In addition to the species shown in the table, *Pinctada*, *Circe*, *Mytilis*, *Spondylus*, and *Mactra* spp. were examined but found to be unsatisfactory because the hearts were either too small or too fragile.

IV. Discussion

The natural habitats of the marine species examined here are estuarine mudflats and are therefore subject to extensive changes in salinity. It may be assumed that the animals possess some degree of resistance to such changes whether by developing increased tolerance of their tissues or by homoregulation. Optimal conditions for heart action probably show minor differences between species; note the different artificial media suggested by Mines (1912), Welsh and Taub (1948), and Gaddum and Paasonen (1955). As the observed differences are relatively small it was considered that sea water should provide an adequate medium for initial survey work.

Prosser and Brown (1961) state that maximum contraction is not obtained unless the heart is distended. In isolated preparations the equivalent of this state is usually achieved by suitable loading of the lever. Greenberg (1960) used an isotonic lever balanced at a load of either 500 or 1000 mg. In the experiments described here the basic load was not determined but was considerably less than either of Greenberg's. The load was increased for certain species with thick-walled ventricles such as *Pinna menkei* and although this never initiated activity it was found to improve the regularity of contraction. In *Fragum unedo* increasing the load increased the sensitivity to serotonin, a stimulant, while in *Regozara flava* the same treatment reduced the effect of adrenaline, a depressant. Thus an increase in load appears to favour activity of the mollusc heart muscle as in the higher animals.

In determining sensitivities of the mollusc hearts to various myotropic substances the effect of earlier treatment with other substances must be considered. Thus, *Regozara flava* ventricle was less sensitive to adrenaline in the presence of serotonin, perhaps not a surprising observation with two substances that produce almost directly opposing actions on this tissue. However, it becomes an important consideration when assessing sensitivities of ventricles such as those of *Cardium setosum* which would only function continuously in the presence of serotonin and therefore probably display abnormally low sensitivity to depressant substances under the conditions of these experiments. It is also probable that serotonin remains adsorbed to the heart tissue for long periods and, certainly, it resists removal by the normal technique of changing the bath fluid. Consequently experiments such as those with *Cardium setosum*, where the ventricle only started to contract rhythmically after stimulation with serotonin, may have given unduly low estimates of sensitivity to adrenaline even though the serotonin had apparently been removed. Such cases may give more accurate results if the optimal conditions are provided by suitable artificial media.

In the only species tested here that consistently responded to adrenaline, *Regozara flava*, there was a slightly greater sensitivity to adrenaline than to noradrenaline. The difference was only twofold and not always evident, so that the preparation has low specificity as judged from present work. This poor differentation of catecholamines by isolated organs of marine invertebrates is the rule rather than

the exception although von Euler, Chaves, and Teodosio (1952) found the pharyngeal sac of *Aplysia dactylomela* was stimulated by noradrenaline and not by adrenaline. Such non-specificity suggests that these catecholamines are not natural mediators in the marine lamellibranchs examined. Although Östlund (1954) reported the presence of amines in the mussel, these amines may be more like epinine than adrenaline or noradrenaline since Hogben and Hobson (1924) showed that *Aplysia* crop responded to epinine in concentrations as low as 0·04 μg/ml while adrenaline was required in concentrations of 10–20 μg/ml.

Data on the effect of catecholamines on mollusc hearts have been considerably increased since the work by Bacq (1947) who stated that invertebrate hearts and tissues show non-specific and usually weak responses to sympathomimetic amines. Fänge (1955) has shown that large concentrations of adrenaline (100 μg/ml) are required to produce an effect on *Anodonta cygnea* heart and Greenberg (1960) required approximately 3·5 μg/ml to elicit a response in *V. mercenaria* heart. In both cases this effect was negatively inotropic. Both Prosser (1940) and Greenberg (1960) stated that low doses of catecholamines had negative inotropic effects on *V. mercenaria* heart and higher doses were positively inotropic. The absence of response to adrenaline found in many mollusc specimens may indicate unsuitable experimental conditions rather than physiological refractoriness. It would seem that the conditions required for maximal sensitivity to a given myotropic substance might be fairly critical and not necessarily the same as those for maximal activity of the isolated tissue.

The sensitivity of *Cardium edule* heart to adrenaline as reported by Gaddum and Paasonen (1955) is approximately the same as that determined for *Regozara flava* and it is interesting that these results indicate a sensitivity equivalent to that of normal mammalian tissues. It is therefore suggested that *Regozara* exhibits at least one of the characteristics required for the assay of adrenaline and noradrenaline, namely adequate sensitivity. This organism has not yet been examined as fully as *Tapes watlingi* with respect to its specificity and reliability (Ladd and Thorburn 1955) nor have attempts been made to obtain increased sensitivity as reported by Ladd (1954) for *Tapes*. Recommendation of this organism as a satisfactory test subject for adrenaline assay must be deferred until more experience has been obtained of its responses under different conditions.

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