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THE ISOLATED HEART OF *MYA ARENARIA* AS A SENSITIVE PREPARATION FOR THE ASSAY OF ACETYLCHOLINE

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The estimation of acetylcholine (ACh) in biological material requires a method of high sensitivity when the concentration likely to be found is very small. Chemical methods so far reported have little value in this respect, for the minimal amount detectable, 0.04 μ.mole in final solution by Hestrin's method (1949), is relatively large. The sensitivity of biological preparations is considerably higher, and the following threshold values have been reported: frog rectus, 2.0 μg./100 ml.; frog heart, 1.0 μg./100 ml.; eserinized dorsal leech muscle, 0.2 μg./100 ml. (Chang and Gaddum, 1933; Gaddum, 1936). In recent years a new method, using the isolated heart of *Venus mercenaria*, has been described. The sensitivity of this preparation to ACh was recorded by Prosser and Prosser (1937), and the application of the method to ACh assay by Wait (1943). Subsequently, many papers have appeared describing the use of this preparation—for example, those of Welsh (1942), Welsh and Taub (1948), Welsh and Slocombe (1952) and Tower and McEachern (1948). The sensitivity and specificity of the *Venus mercenaria* heart are high, and the threshold values for ACh vary from 10^{-1} μg./100 ml. (Smith and Levin, 1938) to 10^{-3} μg./100 ml. (Prosser and Prosser, 1937; Welsh and Taub, 1948).

The method to be described was developed during the course of an investigation into cerebrospinal fluid (c.s.f.) changes in human subjects after head injury. The presence of small concentrations of ACh in the c.s.f. of such cases has been reported by Bornstein (1946), Tower and McEachern (1949) and Ruge (1954). One purpose of this investigation was to test the hypothesis that the beneficial effects of atropine administration in such cases could be linked with the presence of ACh in the c.s.f. (Ward, 1950). The reported concentrations have been small, usually 1–6 μg./100 ml. Clearly some more sensitive preparation than frog rectus or dorsal leech muscle was necessary; Tower and McEachern had recommended the isolated heart of *Venus mercenaria* and had assayed ACh in c.s.f. with this preparation.

Enquiries of Dr. Russell at Plymouth and Dr. Ford at Millport showed that *Venus mercenaria* was only occasionally obtainable and that it could not be supplied regularly or in quantity. Dr. Ford, however, very kindly supplied specimens of various molluscs for trial, and amongst these *Mya arenaria* was found to be the most suitable for size and sensitivity.

METHODS

The method of preparation was essentially the same as that described by Wait (1943), Welsh and Taub (1948), and Tower and McEachern (1948) for *Venus mercenaria*. Slight modifications were made to suit the method to *Mya*.

Regular supplies of *Mya* were obtained from Millport, dispatched by rail packed in seaweed. As soon as they arrived they were stored dry at 4° C. In such conditions they remained alive and active for assay purposes for about two weeks. A few survived for longer periods, but preparations from them tended to be sluggish, irregular in beat, and relatively insensitive.

Mya arenaria is a bivalve mollusc averaging 10 cm. in length. The heart is situated just below the hinge, towards the siphon end (Fig. 1a). When taken from the cold chamber they were left for a few minutes in sea-water at 15° C. The shell was then cracked on both sides by a blow just below the hinge, and that portion of the shell lifted off. The heart could be seen beating within the pericardial sac, which was opened widely. In the *Venus* preparation ligatures are recommended at the auriculo-ventricular junction. With *Mya*, however, this method proved difficult, and it was found much easier to ligate each end of the median ventricle, taking in the intestine and a small portion of the ventricular wall (Fig. 1b). The heart was then cut loose—by dividing the auricles, intestine, and dorsal vessels—and was suspended in a chamber of sea-water. This consisted of an inner portion, filled from below and with overflow emptying; in testing c.s.f., a capacity of 5 ml. to the overflow was found to be a convenient size. The chamber was filled from a sea-water reservoir at 15° C. and surrounded by a water jacket. The heart was suspended from a hollow hook through which air was bubbled. The recording apparatus consisted of a light heart lever ink-writing on a continuous paper kymograph.

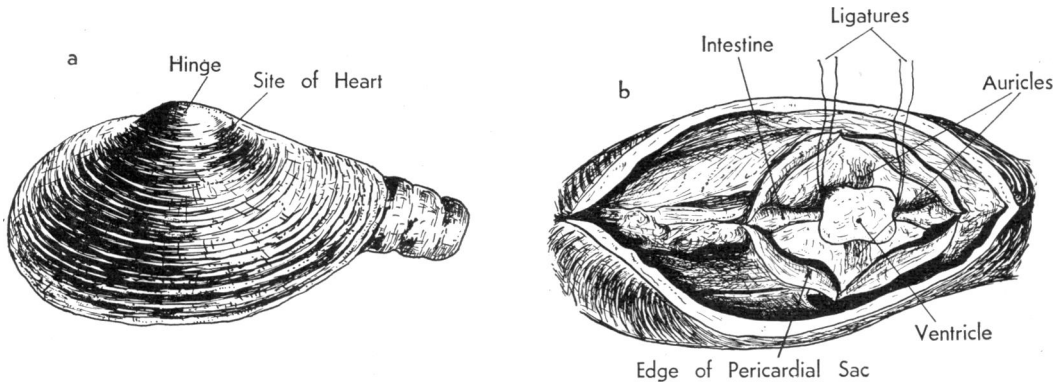

FIG. 1.—Diagrams showing anatomy of the heart region in *Mya arenaria* and the site of application of the ligatures.

A fresh heart started beating within half a minute of setting up ; the beat could be started by tension or by repeated washings with sea-water. In older hearts the beat tended to be slow and irregular at first, but most preparations were beating regularly and ready for use within 15 min. Old hearts that beat irregularly could be revived by the addition of ergometrine maleate. This effect has been described in *Venus* preparations (Welsh and Taub, 1948), and appears to work for *Mya* also. The addition of ergometrine for 2 or 3 min. increased the rate and height of contraction, though sensitivity to ACh was not altered. The ergometrine was then washed out with several changes of sea-water, but the effect persisted for several hours. The preparation could be kept going for 3 to 4 hr. without losing sensitivity : after this period sensitivity to ACh fell off. The test substance was added to the bath with a long hypodermic needle, bent at right angles, on a 1 ml. syringe. After the effect had been recorded the chamber was washed out by overflow with a generous amount of sea-water.

RESULTS

The isolated heart preparation of *Mya* responded to ACh by a reduced amplitude of ventricular contraction, the effect depending on the dose (see Fig. 2). The full effect was not seen for two or three beats of the heart ; thereafter the heart beat at the new level for several minutes and later tended to recover, though seldom to the previous height. As soon as the ACh was washed out the heart recovered quickly, and often beat at a slightly higher level for some time afterwards. Within two or three minutes, however, the preparation again became stable and further tests could be carried out. With large doses the heart stopped immediately in diastole, and after washing there was some delay in recovery.

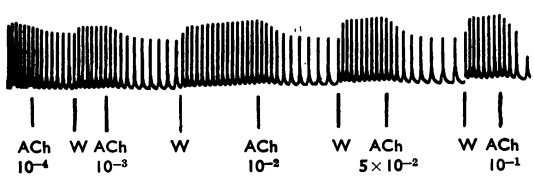

FIG. 2.—Effect of ACh on isolated *Mya* heart. Different doses of ACh (in μg./100 ml.) produce marked slowing of the heart as well as reduction of amplitude. This was an old preparation. The slowing increases as the dose increases.

The response of *Mya arenaria* differs from that of *Venus mercenaria* in that the reduced ventricular contraction may be accompanied by slowing. This was most marked in old specimens, or in old preparations which were losing their sensitivity ; in such preparations slowing might be the only response. This response, however, is not useless, for the degree of slowing is proportional to dose and may be used as a measure of response together with, or in place of, the effect on ventricular contraction.

The sensitivity of *Mya arenaria* to ACh varied a good deal according to the length of storage and time of year. During most of the year the threshold concentration was 10^{-2} to 10^{-3} μg./100 ml. In the months of April and May, however, much greater sensitivity was found in some specimens, and in one batch a response to 10^{-8} μg./100 ml. was obtained.

ACh in very small doses had the effect of increasing slightly the rate and height of ventricular contraction. It was also found that in sluggish hearts after a " stopping " dose of ACh had been administered and washed out the heart beat much better and became more sensitive.

The assay of c.s.f. was done by comparing the effect of doses of ACh with doses of c.s.f. A small chamber was used, for normally only small amounts of c.s.f. were available, the dose used being 0.5 ml. in the 5 ml. chamber. The identity of the test substance was further tested by heating c.s.f. with alkali. After neutralization the previous inhibitory effect was abolished.

Other Effects.—The isolated heart of *Mya arenaria* appeared to be highly specific to ACh, as is the heart of *Venus mercenaria*. Up to 2 ml. of c.s.f. could be added to the 5 ml. chamber without effect, and c.s.f. containing protein up to 4 g./100 ml. could be added in 0.5 ml. doses without effect. Human blood in 1 ml. doses had no effect.

Noradrenaline in doses up to 100 μg./100 ml. had no effect. With larger doses the heart rate increased and tonus increased.

The effects of methacholine, and of benzoylcholine, were essentially similar to that of ACh, but the preparation was considerably less sensitive, the threshold for methacholine being about 10,000 times that for ACh.

Temperature had a marked effect on this preparation. Below 10° C. the heart beat was slow and of low amplitude and the sensitivity to ACh was greatly reduced. Above 20° C. the beat was slow and irregular; again ACh sensitivity was reduced. With *Venus* preparations an optimum temperature of 15° C. was recorded; with *Mya* a temperature slightly above this was found to be more favourable, and the best results were obtained at 17°–18° C.

Discussion

The isolated heart of *Mya arenaria* is a highly specific preparation for the assay of acetylcholine. It compares favourably in this respect with *Venus mercenaria* and is easily obtainable in this country. It is slightly more sensitive than *Venus* at most times of the year, and in the spring considerably more so. It has the disadvantage that the reduced ventricular contraction is sometimes accompanied by slowing of rate, but as the slowing is proportional to the dose this can be used as a measure of response.

Summary

1. In the assay of acetylcholine (ACh) in cerebrospinal fluid (c.s.f.) there is need for a highly specific and sensitive preparation. *Venus mercenaria* is such a preparation, but is not obtainable everywhere.

2. The method of preparation and use of the isolated heart of *Mya arenaria*, which is easily obtainable in the British Isles, are described. This heart has a high specificity and sensitivity to ACh, to which it responds by reduction of the amplitude of ventricular contraction with or without slowing. The degree of inhibition or slowing is proportional to the dose of ACh. The threshold concentrations usually lie between 10^{-2} and 10^{-3} μg./100 ml.

3. No response is obtained from large doses of normal human c.s.f., highly proteinous c.s.f., or human blood. The effect of ergometrine maleate is to increase the rate and height of ventricular contraction without altering sensitivity to ACh. The effect of noradrenaline is to increase the rate and tonus.

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