EXAMINING THE EFFECTS OF NEUROTRANSMITTERS ON CLAM HEARTS

BIO 346 NEUROBIOLOGY

INTRODUCTION

Mollusk hearts, like those of vertebrates, are **myogenic**, i.e. contraction is inherently rhythmic and occurs spontaneously in the cardiac cells. If the heart is severed from nervous connections, removed from the body, or even cut into pieces it will continue its periodic contractions for some time. Cardioregulator nerves, however, modify the frequency and amplitude of the beat through the release of neurohumoral agents. In most mollusks, as in vertebrates, acetylcholine is the mediator of the inhibitory fibers to the heart. In other words when the inhibitory fibers to the heart are stimulated, acetylcholine is released from their terminals, diffuses to receptors on the cardiac muscle and causes the activity in the cardiac muscle fibers to decrease. Unlike the vertebrates, the excitatory transmitter for mollusk hearts is 5-hydroxytryptamine (5-HT, serotonin). (In the vertebrates epinephrine or norepinephrine is released by cardioexcitor nerves.) The heart of some clams is so sensitive to ACh and 5-HT that it is often used to assay other biological tissues for these compounds. In today's experiment you will examine the effects of several compounds on heart contraction of the quahog or cherrystone clam, *Mercenaria mercenaria*..

PROCEDURE

Dissection and Preparation

To expose the heart, break and remove the dorsal portion of the shell (umbo: dorsal protuberance- and hinge). The dissection will be demonstrated. Then cut away the mantle and pericardium dorsal to the heart. The heart consists of a single, median ventricle with thin-walled atria (auricles) projecting laterally. (See figure 1.) Clam anatomy is a little unusual in that the intestine goes directly through the ventricle!

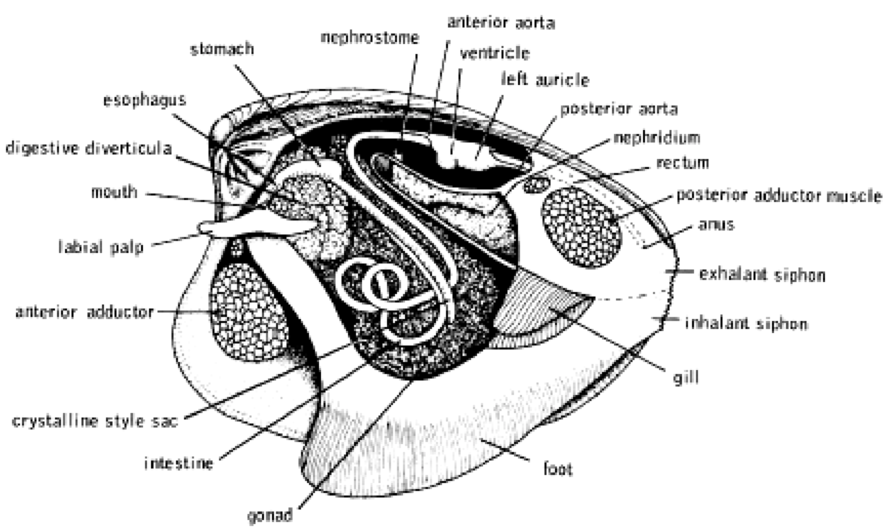

FIGURE 1. Schematic diagram of clam anatomy.

Slip the tips of a pair of curved forceps underneath the junction between one of the auricles and the ventricle. Open the forceps slightly and grasp the tip of a short length of thread. Pull the thread through and tie a square knot around the proximal portion of the auricle leaving the junction itself intact. Leave a fairly long length of thread attached (≅ 9 inches) (see figure 2).

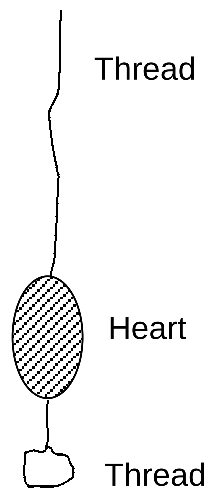

FIGURE 2. Diagram of heart preparation.

Prepare another piece of thread by tying a small loop at one end. Use it to tie a knot around the other auricle thereby attaching the loop. Cut through the auricles distal to the knots and sever the arteries and the gut anterior and posterior to the ventricle (see figure 2). The ventricle is now free and can be removed (with the proper lengths of thread attached) and placed in the muscle bath. (figure 3)

The muscle chamber should be set up so that it is connected to a sea water reservoir and air is continuously being bubbled in through a needle placed through the rubber septum.

Examine the muscle chamber and determine where all the tubes go and how sea water flows through the chamber. There is an outer jacket which can be used to change the temperature of the bath.

Mounting the Heart

The heart holder consists of a rod through which a thin piece of glass rod is mounted perpendicularly. The glass rod is bent into an S at one end. Place the heart holder above the chamber which should be filled with sea water. (The tubing attached to the bottom of the bath should be clamped off to keep the sea water in the bath.) Now take the loop which you attached to one end of the heart and place it over the S hook of the glass rod (Figure 3). Attach the thread tied on to the other end of the heart to the transducer arm which is mounted on the ringstand above the muscle chamber. The thread can be attached with wax or modeling clay. Raise the muscle chamber up on the ringstand so that the S-hook is as low as possible in the chamber without touching the sides. The other end of the transducer arm should be balanced so that there will be a slight tension on the heart. This can be accomplished by adding wax or clay to the arm. (figure 3). You are now ready to begin recording heart beat.

Recording the Heart Beat

The transducer is connected to an amplifier, the output of which is led into the computer data acquisition system. The computer will simulate a chart recorder. Open the CLAM.ACQ from the Acknowledge program..

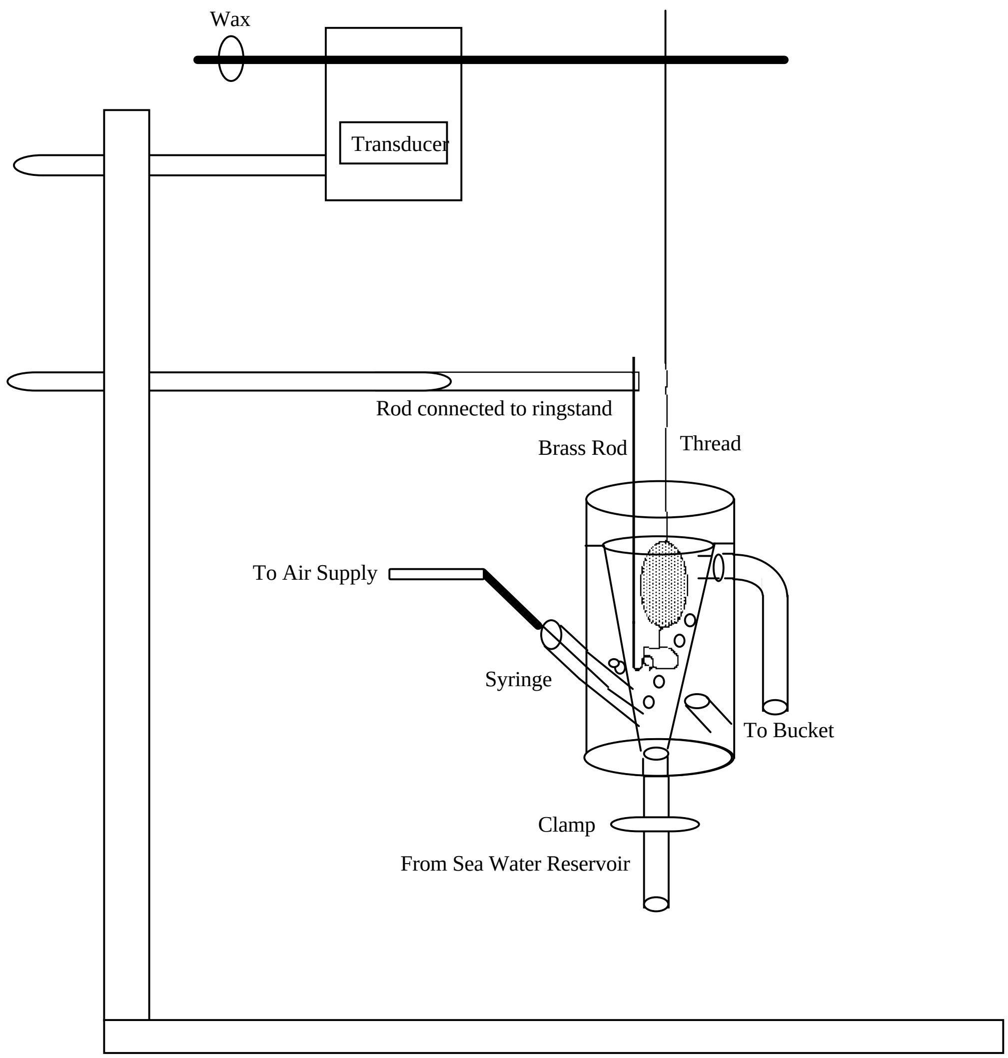

FIGURE 3 Schematic diagram of the set up.

Experiments

You will be supplied with several compounds including acetylcholine and 5-HT. As already mentioned, ACh slows down heart rate and 5-HT speeds it up. A stock solution has been prepared for both of these compounds (ACh, 10^{-2}M: 5-HT, 10^{-2}M). You will need to dilute them before you deliver them to the muscle chamber. They will be added to the bath in 50µl aliquots. Since the volume of the bath is ≅ 6ml, a solution added to the chamber will be diluted by approximately 100 times. That is, a 10^{-3}M solution will be diluted to ≅ 10^{-5}M when added to the bath.

To add solution to the chamber, draw 50 µl into the pipette which is then carefully placed into the sea water bathing the heart. Squirt the solution into the chamber, without touching the heart. The air bubbles help in the mixing process and within a few seconds the heart will be bathed in the test solution.

To remove the test solution, remove the tubing clamp at the bottom of the chamber and allow fresh sea water to bathe the heart for about 10-15 seconds. The heart beat should return to normal.

You can obtain dose-response curves for ACh and 5-HT. Begin with concentrations around 10^{-9}M (after dilution) and increase in 1 log unit steps.

Once your group has become proficient in recording from the clam heart, you should decide on what experiment you would like to do in the following three weeks. A number of chemicals can be made available if you would like to determined what kind of channels or receptors might be involved. Whatever your group decides, please give us enough time to obtain the materials you will need to conduct your experiment.

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