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THE EFFECT OF 5-HYDROXYTRYPTAMINE
ON THE MAINTENANCE OF TONICITY AND RHYTHMIC ACTIVITY
OF THE ADDUCTOR MUSCLES OF FRESHWATER MUSSELS

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By

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ABSTRACT

THE EFFECT OF 5-HYDROXYTRYPTAMINE
ON THE MAINTENANCE OF TONICITY AND RHYTHMIC ACTIVITY
OF THE ADDUCTOR MUSCLES OF FRESHWATER MUSSELS

By

George H. Conover, III

The effects of 5HT on active-rest periodicities and rhythmic frequencies and tonicities were examined in several freshwater mussels (Anodonta grandis, Amblema costata, Eliptio complanatus, Eliptio dilatatus) by recording valve displacements. Electrical activity was recorded simultaneously from both adductor muscles.

Active periods are characterized by a separation of the valves accompanied by short rhythmic contractions and associated bursts of electrical activity as recorded from both adductors. 5HT induces an active period when injected into either adductor during a rest period and causes an increase in valve separation and in the rate of relaxation following each rhythmic contraction when injected during an active period.

5HT also increases the relaxation rate, under a constant load, of isolated posterior adductor preparations and of small bundles of fibers obtained from this muscle. 5HT had no significant effect on isolated muscle preparations when the visceral ganglion was included or when the muscle received d.c. stimulation.

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I am grateful to my major advisor, Dr. Ralph A. Pax, for providing guidance and good humor throughout the course of this study and to Drs. Gerard L. Gebber and Charles D. Tweedle who served as members of my graduate committee.

This acknowledgment would not be complete without also mentioning the future neurophysiologists, the optometrist, the veterinarian, and the tenor sax man who intermittantly served as fellow graduate students and who shared with me many good times.

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INTRODUCTION

Muscles exhibit a variable but distinct resistance to stretch following actin-myosin interaction. Molluscan muscles, in particular, have capitalized on this characteristic to the extent that they can remain in the contracted state for several hours or even days. This sustained contraction has been termed "catch" and muscles capable of this type of contraction are called catch muscles. Catch is characterized by an absence of action potentials (Fletcher, 1937, Jewell, 1959, Johnson and Twarog, 1960, Twarog, 1967) and an expenditure of energy no more than ten percent greater than that of the resting muscle (Baguet and Gillis, 1968, Nauss and Davies, 1966).

The Anterior Byssal Retractor Muscle

Types of Contractions

Most of what is known of the electrical, neurochemical and metabolic aspects of catch muscle has come from work on the anterior byssal retractor muscle (ABRM) of Mytilus, a marine mussel. The ABRM is capable of two distinct types of contractions. The first, the phasic response, can be produced by a.c. pulses applied directly to the muscle or to the cerebro-pedal connective. It is characterized by an increase in tension which is maintained for the duration of the stimulus but which rapidly relaxes after the stimulus is

terminated.

The second type of contraction, the catch or tonic response, can be produced in the same muscle by d.c. current, acetylcholine (Ach) (Twarog, 1954, 1960, Hidaka and Goto, 1973) or high potassium media (Tameyasu and Sugi, 1976, Sugi and Yamaguchi, 1976, Muneoka, 1974). The development of tension is similar to that in the phasic contraction but the increased tension is maintained, relaxing only very slowly after the stimulus is terminated.

Neurotransmitters

5-hydroxytryptamine (serotonin, 5HT) and to a lessor extent 3-hydroxytyramine (dopamine) and some ergot alkaloids, when applied to an ABRM in catch, cause an almost immediate loss of tension in the muscle (Hidaka, 1969, Hidaka and Twarog, 1967, Twarog, 1967). Following treatment with 5HT, the ABRM remains capable of contracting when stimulated electrically with d.c. current or chemically with Ach but all contractions are of the phasic type. Thus 5HT affects only the ability to maintain tension following a contraction.

5HT is present in the central nervous systems of mussels and other pelecypods and gastropods (Welsh, 1960, Dahl, 1966, Zs Nagy, 1967, Hiripi, 1968). In addition, 5HT is released from nerve terminals into the ABRM during electrical stimulation of the cerebro-pedal connective (York and Twarog, 1972). Thus 5HT may be the neurotransmitter involved in the release of catch.

Mersalyl, a presumed 5HT blocker, prevents the release of tension due to 5HT so that when the ABRM is treated with both mersalyl and 5HT, the relaxation rate following a catch contraction is similar to

that of an untreated muscle (Twarog et. al., 1977). The release of tension due to dopamine is also blocked but only at much higher concentrations of mersalyl. Thus the effect of mersalyl is specific in preventing the catch releasing effect of 5HT. The blocking action of mersalyl can be reversed by the addition of dithiothreitol (DTT) and reversal occurs at a rate many times faster than that due to simple washout of the mersalyl in normal saline. Twarog et. al. (1977) conclude that mersalyl blocks the action of 5HT at or near the receptor site for 5HT.

5HT may increase the intracellular cAMP level by either activating adenylate cyclase or by inhibiting phosphodiesterase (Higgins and Greenberg, 1974, Higgins, 1974, Cole and Twarog, 1972). cAMP directly induces relaxation and release of catch but only when the muscle membrane has been chemically treated with EDTA or triton X-100 to increase the permeability of the muscle fiber bundles (March-and-Dumont and Baguet, 1975).

Gilloteaux (1972, 1977) has identified two types of nerve endings within an ABRM muscle bundle, one containing clear vesicles which he identified as being cholinergic and another more numerous type of ending containing dense-cored vesicles which he tentatively identified as being tryptaminergic.

Role of Calcium

Considerable work has been done on the role of Ca^{++} in the activation of the ABRM (Sugi and Yamaguchi, 1976). A Ca^{++} free medium will immediately reduce the degree of tension developed when the muscle is stimulated by Ach or high potassium, two treatments which normally

put the ABRM into catch. The response of the muscle to either stimulus is abolished within 20 to 30 minutes after placing the muscle into a Ca^{++} free medium although the membrane potential is unaffected for up to one hour. Low Ca^{++} media affect only the potassium contracture, however. The Ach contracture is reduced by procaine, a compound known to inhibit the release of Ca^{++} from the sarcoplasmic reticulum of vertebrate muscles (Muneoka, 1969) although the potassium contracture is unaffected by procaine. Sugi and Yamaguchi (1976) conclude that high potassium may act to increase Ca^{++} influx into the muscle cell while Ach causes the release of intracellular Ca^{++} stores and is thus more inhibited by procaine. Both high potassium and Ach increase the concentration of intracellular free Ca^{++} and hence initiate a contraction.

The Ach contracture is relaxed at lower concentrations of 5HT than those necessary to relax the potassium contracture. Sugi and Yamaguchi (1976) suggest that 5HT may reduce the level of intracellular Ca^{++} by selectively increasing the uptake of Ca^{++} by intracellular storage vesicles. Atsumi et. al. (1976) located such Ca^{++} accumulating vesicles along the inner cell membrane. The contents of these vesicles are released into the myoplasm during an active contraction following the application of Ach. During relaxation, the Ca^{++} is taken up by the peripheral vesicles leaving very little free Ca^{++} in the myoplasm. Similar effects are seen in relaxations produced by 5HT. These data are generally compatible with measurements of Ca^{++} influx and efflux as measured by several authors (Hagiwara, 1970, Huddart et. al., 1977, Muneoka and Mizonishi, 1969) although some authors (Bloomquist and Curtis, 1975a and b) have suggested that the source(s) of the

intracellular Ca^{++} accompanying Ach application and the decrease of free Ca^{++} with 5HT application may be considerably more variable and complex.

The Valve Adductors

Anatomy

The valve adductors are a second, less intensely examined class of molluscan muscles described as displaying catch. Most of the more recent work on adductors has centered on the fresh water mussel, Anodonta (Salanki, 1963, 1966, Salanki and Varanka, 1972) and the marine bivalves Crassostrea (Millman, 1963, 1964), Mytilus (Lowy, 1953), and Pecten (Mellon, 1968, 1969, Millman, 1976). In most of the species examined, the valves are adducted by two muscles, an anterior and posterior adductor, each of which is composed of a translucent (yellow) portion and an opaque (white) portion neither of which is cross striated. A notable exception is the scallop, Pecten, which possesses a single adductor composed of a cross striated portion and an opaque portion but lacking a translucent portion. The unique cross striated muscle of Pecten presumably enables the swimming behavior displayed by this animal.

In those species possessing paired adductors, there is considerable variability in the relative cross sectional areas of translucent as compared to opaque portions (Salanki and Zs Nagy, 1966). Opaque and translucent muscles have been assumed to represent muscles capable of tonic and phasic contractions, respectively (Bullock and Horridge, 1965). Isolated opaque portions of the adductor muscle of Crassostrea are similar to the ABRM in that they respond to Ach with a tonic contraction

resembling catch (Millman, 1964). Salanki and Zs Nagy (1966), however, point out that although three of the marine lamellibranchs, Cardium, Ensis, and Mytilus, all contain almost exclusively translucent muscle, Cardium and Ensis are incapable of maintaining a tonic contraction whereas Mytilus is capable of tonic contractions lasting several days.

Both translucent and opaque adductor muscle fibers contain thick and thin myofilaments. The thin filaments contain actin while the thick filaments contain myosin and paramyosin. Translucent and opaque fibers have similar numbers of thick filaments per cross sectional area and both thick filaments are similar in length. Thick filaments within the opaque fibers, however, have larger average diameters than those in the translucent fibers (Zs Nagy et. al., 1971). Thin filaments from translucent and opaque fibers are similar in all measured parameters.

Structural Basis of Catch

Two hypotheses have been proposed to account for the structural basis of catch in the ABRM and adductor muscles. The first of these proposes that, at the time the muscle enters into catch, the paramyosin within the thick filaments undergoes a structural change, interacting with adjacent thick filaments, to form a complex which is highly resistant to stretch (Johnson et. al., 1959, Zs Nagy et. al., 1970, 1971, reviewed by Ruegg, 1971). The maintained catch tension would, thus, be independent of the presumed actin-myosin interaction which accounts for the active contraction.

The alternative explanation, the "linkage hypothesis" first proposed

by Lowy et. al. (1964), accounts for catch in terms of actin-myosin interactions between the thick and thin filaments. The cross linkages thus formed either break very slowly (Lowy and Millman, 1963) or continuously break and reform for the duration of the catch state (Baguet and Gillis, 1968). The rate at which these linkages are broken may be controlled by the level of 5HT in the muscle. Recent ultrastructural evidence on the nature of filament interactions (Nonomura, 1974, Epstein et. al., 1975) and X-ray diffraction studies (Millman and Elliot, 1972) have tended to favor the "linkage hypothesis" since no structural differences have been found in either thick or thin filaments in catch as compared to the relaxed state.

Characteristic Activity

Most bivalves display alternating periods of "rest", in which the adductors are tonically contracted with the valves tightly closed and "active" periods in which the valves are separated and the adductors relaxed to some degree (Marceau, 1909, Barnes, 1955). Each of these periods often lasts several hours with the active period usually being significantly longer lasting. There is extreme variability in the average duration of each period and in the ratio of the average duration of active periods to rest periods (Salanki, 1970). The active-rest periodicity appears to be independent of diurnal factors (Barnes, 1955, Salanki, 1971) although it can be modulated by environmental factors, particularly oxygen level (Salanki, 1965, 1967, Badman, 1974) and interoceptive stimuli (Salanki, 1962).

Superimposed on the slow periodicity during the active phase, is a faster rhythmicity consisting of quick contractions of the adductor

muscles. These contractions have a variable frequency of from less than one to fifteen or more per hour (Lowy, 1953, Barnes, 1955, Salanki, 1970, 1971). The rhythmic contraction frequency is typically highest at the initiation of the active period, gradually decreasing to a minimum and then again rising prior to the termination of the active period (Salanki, 1970).

The rhythmic contractions are correlated with bursts of electrical activity in both the visceral ganglia and the cerebro-visceral connective. This activity precedes the contractions by 0.5-1.5 seconds (Salanki and Varanka, 1972). Bursting activity has been recorded from isolated visceral ganglia and rhythmic contractions of the adductor muscles cease when the visceral ganglia are removed (Salanki, 1967, Salanki and Varanka, 1972). Salanki and Varanka have thus concluded that the visceral ganglia have a triggering influence on rhythmic activity although the rhythm can be altered by environmental factors.

Lowy (1953) recorded bursts of electrical activity in the adductor muscles of Mytilus which were synchronous with valve movements. Similar electrical activity often extended into the subsequent rest period although the rhythmic contractions of the adductors were only recorded during an active period. More recently, simultaneous recordings of the electrical activity in the two adductors of Anodonta have been made (Labos et. al., 1968). It has been found that, in quick contractions, the electrical activity in the two adductors is not synchronous but that activity in the anterior adductor precedes that in the posterior by an average of 280 msec.

Catch and the Release of Catch

5HT appears to have a catch releasing role in the adductors similar to that in the ABRM. It causes an immediate relaxation of catch when injected into the posterior adductor muscle (Salanki and Labos, 1969, Salanki, 1963, Millman, 1964, Puppi, 1963) and relaxation within one minute when applied to the cerebral ganglion (Salanki, 1963). The 5HT level in the anterior adductor is twenty-five to thirty percent higher at the beginning of an active period while that of the visceral ganglion is twenty-three percent lower. Relaxation of both adductors appears to parallel the increase in 5HT concentration (Salanki and Hiripi, 1970, Salanki et. al., 1968). 5HT carboxylase, necessary for 5HT synthesis, has been found in the CNS (Welsh, 1959) but has not been detected in the adductors (Hiripi and Salanki, 1969). Salanki et. al. (1968) suggest that 5HT may be synthesized in the ganglia, released and transported via the cerebro-visceral connective to the adductor muscles where it appears to influence the release of catch and the onset of an active period.

While there is considerable evidence that 5HT has similar effects on the ABRM and the valve adductors, there is considerable ambiguity as to the effects of Ach and dopamine on the adductors. In the ABRM, Ach is excitatory while dopamine has an effect similar to that of 5HT. Ach administered to isolated opaque adductor in Crassostrea also causes an excitatory response (Millman, 1964). However, the rate of tension development is much slower and the percent of maximum tension developed less than that in the ABRM. It is necessary that the visceral ganglion be left intact and that magnesium be replaced by

Ca^{++}in the medium in order to get a maximal response.

In Anodonta, Ach causes relaxation of the adductors when it is injected into intact animals but produces an excitatory response when the cerebro-visceral connective is severed (Puppi, 1963). Dopamine causes a tonic contraction of the adductor muscles of Anodonta (Salanki and Labos, 1969). Dopamine has been proposed as a catch inducer by the same authors (Salanki et. al., 1974) who measured a thirty percent decrease in the concentration of dopamine in the cerebral and visceral ganglia corresponding to the onset of a rest period. It should be noted that this effect is the opposite of that observed in work on the ABRM where dopamine is effective in decreasing tonicity and releasing catch.

Objectives of the Present Study

The present study is an attempt to further elucidate the physiological and chemical characteristics of the long term periodicity and rhythmic activity of the valve adductors of several fresh water bivalves. The adductors have been chosen because: 1. They are particularly specialized in the tonic catch contraction yet also demonstrate spontaneous phasic activity allowing for the comparison of characteristics. 2. They can be recorded from with minimal disruption to the intact animal. The majority of the studies on catch mechanisms have concentrated on the ABRM which had been removed from the animal and suspended in saline. The adductors can be reached easily by means of holes drilled through the valve for the placement of electrodes. Thus recordings can be made from an animal with neural and circulatory channels intact. 3. In addition, observations can be made of the behavior of the mussel, for example, foot protraction, resulting in a more integrated

view of valve adduction as correlated with the animal's overall behavior.

The general aim of the present study is to first examine the naturally occurring periodicities and rhythmicities and, second, to observe and quantify the alterations of these activities due to the influence of 5HT. Many questions have been raised concerning the adductors in previous work. Specifically, little is known of the effect of 5HT on the rhythmic contractions, although the sensitivity of the muscle to a.c. stimulation was said to be increased resulting in a greater valve adduction with each contraction (Millman, 1964, Salanki and Hiripi, 1970). The coordination of the two adductors in phasic as well as catch contractions has not been extensively studied. Examination of the effect of 5HT and other chemicals has been concentrated on the posterior adductor. Little is known about their effects on the anterior adductor. Electrical activity has been recorded from the adductors but there is disagreement as to whether specific types of electrical activity are associated with catch and rhythmic contractions (Lowy, 1953, Labos et. al., 1968). The effect of 5HT on this electrical activity has not been examined. Finally, only a single author has been successful in studying the physiology of an isolated adductor muscle but only after altering certain normal conditions, for example, replacing magnesium with Ca^{++} in the bathing medium (Millman, 1964). The following study was undertaken in order to clarify aspects of these problems.

MATERIALS AND METHODS

Source and Maintenance of Animals

Fresh water mussels of the species Anodonta grandis, Amblema costata, and Eliptio complanatus were collected locally in South Lake and the Thornapple River in southern Michigan. Additional mussels (Eliptio dilatatus delicatus) were purchased from Carolina Biological Supply, Co. All animals were maintained in the laboratory at room temperature (20-23°C.) in aerated tap water. The water in which the mussels were stored was changed at least once a week. Freshwater mussels could be maintained in this manner for several months although most were used within 2-3 weeks of collection or purchase. All experiments were performed at room temperature.

Whole Animal Experiments

Preparation

The mussel was placed into a 2 liter glass vessel containing aerated tap water. The left valve was clamped securely into place while the right valve was connected by way of a lever system to a Narco Biosystems A-1415 myograph transducer. In this way, valve movements could be continuously monitored on a Narco Biosystems Physiograph. Under natural conditions, the adductors must maintain tension against a constant opposing force, the hinge ligament, which tends to open the valves. Experiments were done to determine the effect of increasing this force

by placing an additional load on the muscle. This resulted, however, in a significant alteration of valve movements. Both the long term periodicity and the short rhythmic contractions were affected. For this reason, all experiments were performed with minimal load added to the muscle.

In preparation for recording electrical activity in the adductor muscles, two small holes were drilled through the left valve in the area of both the anterior and posterior adductors and two insulated 34 gauge silver electrodes were inserted into each of the adductors and glued into place with Woodhill E.POX.E 5 cement. The electrical activity was amplified by way of a Grass P15 Preamplifier or a Narco 7171 High Gain Coupler and Channel Amplifier and displayed on separate channels of an oscilloscope and physiograph. Differential recordings from each of the two adductors were then displayed simultaneously against the mechanical displacements.

Additional holes were then drilled through the left valve in the vicinity of the anterior and posterior adductors and lengths of polyethylene tubing (I.D. .045" - O.D. .062") were inserted approximately 3 mm. into each muscle and glued into place. Chemicals were dissolved in freshwater mussel saline (Potts, 1958) and injected directly into the muscle by way of this tubing.

Procedure

Mussels were prepared as described and placed into aerated water in an orientation closely resembling their natural position. Several long term (1-5 day) recordings of spontaneous valve movements were made to determine whether activity differed appreciably over time. This

was not found to be significant following a short lived, 1-2 hour, initial period of adjustment. Subsequent activity remained fairly constant over the next several days.

All experimental animals were allowed 2-3 hours to equilibrate prior to the injection of drugs. After this period, a single injection of saline (.5 ml.) was administered into either the anterior or posterior adductor, or into the mantle cavity, depending on the particular experiment. This was followed by sequential injections of 5HT at concentrations of 10^{-7} M., 10^{-6} M., and 10^{-5} M. A minimum of 30 minutes was allowed between injections or until the valves returned to control positions and the rate of rhythmic contractions returned to pre-injection levels. Measurements were made of the frequency of rhythmic contractions, the rate of relaxation following each rhythmic contraction and the degree of separation of the valves as compared to pre-injection levels. Observations were made of behavioral changes throughout the experiments.

The experimental groups were as follows:

1. Control- received sequential injections of freshwater saline. (N=2)

2. Experimental group I- received an initial injection of saline followed by sequential injections of 5HT into the mantle cavity. (N=5)

3. Experimental group II- received an initial injection of saline and sequential injections of 5HT into the anterior adductor muscle. (N=6)

4. Experimental group III- received an initial injection of saline and sequential injections of 5HT into the posterior adductor muscle. (N=6)

Isolated Whole Muscle Experiments

Preparation

For isolated muscle studies, two groups of preparations were used. The first was a ganglion-muscle preparation in which the visceral ganglion was left intact and attached to the ventral surface of the posterior adductor muscle. All afferent and efferent nervous connections were cut except those supplying the muscle itself. The second preparation differed in that the ganglion was removed. In both cases, the muscle was dissected free of surrounding tissue and placed into a 30 ml. paraffin dish containing mussel saline. Small fragments of shell were left attached to each end of the the muscle. Holes were drilled in these shell fragments so that the muscle could be anchored to the dish at one end and connected by a short thread to the myograph lever system at the other end.

Isolating the muscle in this way removed the hinge ligament so that it was necessary to connect an additional load to the system. A variety of loads was tested, ranging from 8 g. to 100 g., in order to find the load which was sufficiently heavy to record a relaxation of the muscle without inhibiting normal maintained (catch) tension. For most of the isolated muscle experiments, a standard load of 38 g. was used.

Introduction of chemicals into this preparation was by either direct injection into the muscle or by injecting sufficient amount of the chemical into the medium to bring it to the desired concentration. The former method proved to be more effective in the isolated whole muscle studies.

In experiments where d.c. stimulation was applied, two 34 gauge

silver electrodes insulated to within 2 mm. of their tips, were inserted into the muscle following the mounting of the muscle in the dish. 30 volt d.c. stimulation was applied for a period of 60 seconds.

Procedure

Isolated muscle and muscle-ganglion preparations were dissected out and mounted in the paraffin chamber containing freshwater mussel saline. Half of the isolated muscle preparations (N=5) were stimulated with d.c. current (30 V.) for 60 seconds before being loaded. The remainder of the isolated muscle preparations (N=5) as well as the muscle-ganglion preparations (N=5) received no stimulation.

Each preparation was then loaded with a 38 g. load. This resulted in an initial rapid stretch which quickly slowed to a constant rate of relaxation. Although this slower rate was normally reached within about 20 minutes, a full 60 minutes was allowed to enable the muscle to fully equilibrate. A .3 ml. direct injection of 5HT at a concentration of 10^{-7} M. was administered at this time and the effects on the rate of relaxation of the muscle were determined and compared to that prior to injection. In some instances, this was followed, after 30 minutes, by a second injection of 5HT of equal volume but at a concentration of 10^{-6} M.

Isolated Muscle Bundle Experiments

Preparation

The posterior adductor muscle was removed and dissected free of ganglia and connective tissue. A small bundle of muscle fibers, 2-3 mm. in diameter, was obtained by carefully teasing apart the fibers in the opaque portion of the muscle, leaving each end of the muscle bundle attached to a shell fragment as previously described. The muscle was placed into a 30 ml. dish containing freshwater mussel saline and was attached to the myograph transducer. A load of 25 g. was used for this series of experiments. Drugs were administered in all cases by addition to the bathing medium. Mechanical displacements of the muscle were recorded on the physiograph.

Procedure

Muscle bundles were prepared and mounted in the paraffin chamber containing mussel saline. The bundle was loaded with 25 g. and, following a period of equilibration (30 minutes), was exposed to 10^{-7} M. 5HT. An additional 30 minutes was allowed for the muscle to equilibrate and this was followed by an increase in the concentration of 5HT in the medium to 10^{-6} M. As before, the effects on rate of relaxation were monitored.

RESULTS

Behavioral observations of freshwater mussels indicate that there are two distinct periods of different relative valve separations: an "active" period in which the valves remain separated to a variable degree and a "rest" period in which the valves remain tightly closed, sometimes for extended periods of time. The relative durations of each of these periods are quite variable (30 minutes to 8 or more hours) although the periods tend to be more regular within a particular animal than between animals. This alternation of activity and rest will be referred to, in this paper, as the slow periodicity.

Several long term (2-5 day) recordings of valve displacements were made from a group of mussels (N=8) to determine the normal slow periodicities. Figure 1 illustrates some examples of the types of activity recorded. Figure 1A is a graph of the valve displacements taken from a mussel over a 30 hour period. In this particular animal, the rest periods were intermediate in length (1-3 hours) while the active periods tended to be somewhat longer lasting (3-6 hours) and to increase in length over the time in which the recordings were made. Figures 1B and 1C are similar graphs for two other animals and indicate the high degree of variability in periodicities among different animals. The mussel represented by figure 1B had longer lasting rest periods (40-60 minutes) as compared to the active periods (30-40 minutes), with

Figure 1. Valve displacements during the slow periodicity. 0 valve separation indicates a rest period. Active periods are intervals in which the valves are separated to a variable degree. Three typical patterns of activity are shown. In A the rest periods are intermediate in length (1-3 hours) while active periods tend to be somewhat longer lasting (3-6 hours). In B both active and rest periods are more brief than in A, the rest periods being longer lasting (40-60 minutes) than the active periods (30-40 minutes). In C the subject entered into a single long lasting active period. A and C each represent 30 hours of recording time while B represents 5 hours.

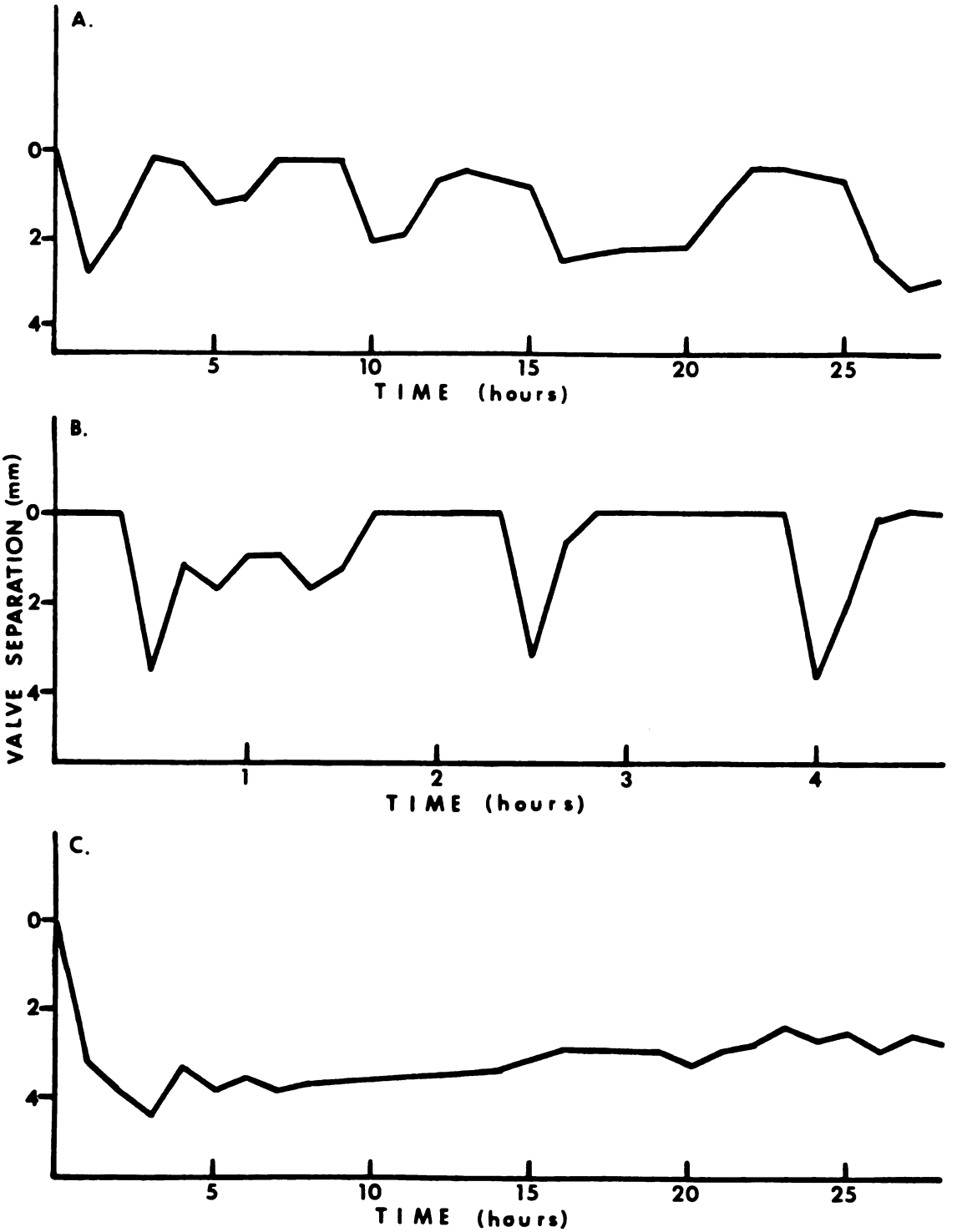
A.
0
2
4
5
10
15
20
25
TIME (hours)
B.
VALVE SEPARATION (mm)
0
2
4
1
2
3
4
TIME (hours)
C.
0
2
4
5
10
15
20
25
TIME (hours)

Figure 1.

the active periods remaining fairly consistent in duration (about 30 minutes). Figure 1C represents an animal which entered an active period and remained active for the duration of the observation period. The amount of separation of the valves during active periods tended to be fairly constant within an individual but was highly variable between individuals.

The beginning of an active period is marked by an increase in separation of the valves. At this time, there also occurs a series of rhythmic contractions. These contractions cause valve adductions, the magnitudes of which are dependent on the degree to which the valves were separated prior to the contractions. Larger valve separations allow for a greater amount of adduction with each contraction although the adductions are typically sub-maximal. Figure 2 is a recording of a series of rhythmic contractions during an active period.

Also shown in figure 2 are electrical recordings taken from both adductors simultaneously with the valve displacements. Each contraction is marked by a burst of electrical activity as recorded from both the anterior and posterior adductors. Rhythmic contractions could not be observed during a rest period because the valves were already tightly closed but electrical bursts of this type were still recorded for a short time after an animal had entered into a rest period. The bursts were identical to those accompanying the rhythmic contractions during an active period although the intervals between contractions were longer. In animals with longer lasting rest periods, bursts were only rarely seen except at times just prior to the initiation or just following the termination of an active period.

Figure 2. Rhythmic contractions and associated electrical activity during active and rest periods.
Upper trace: electrical activity of the anterior adductor muscle. Vertical calibration: 0.5mv.
Middle trace: electrical activity of the posterior adductor muscle. Vertical calibration: 0.5mv.
Lower trace: valve displacements. Vertical calibration: 2.0 mm.

The recording begins with the animal in rest, continues with entrance into an active period and ends with the subsequent rest period.
Horizontal calibration: 2 minutes.

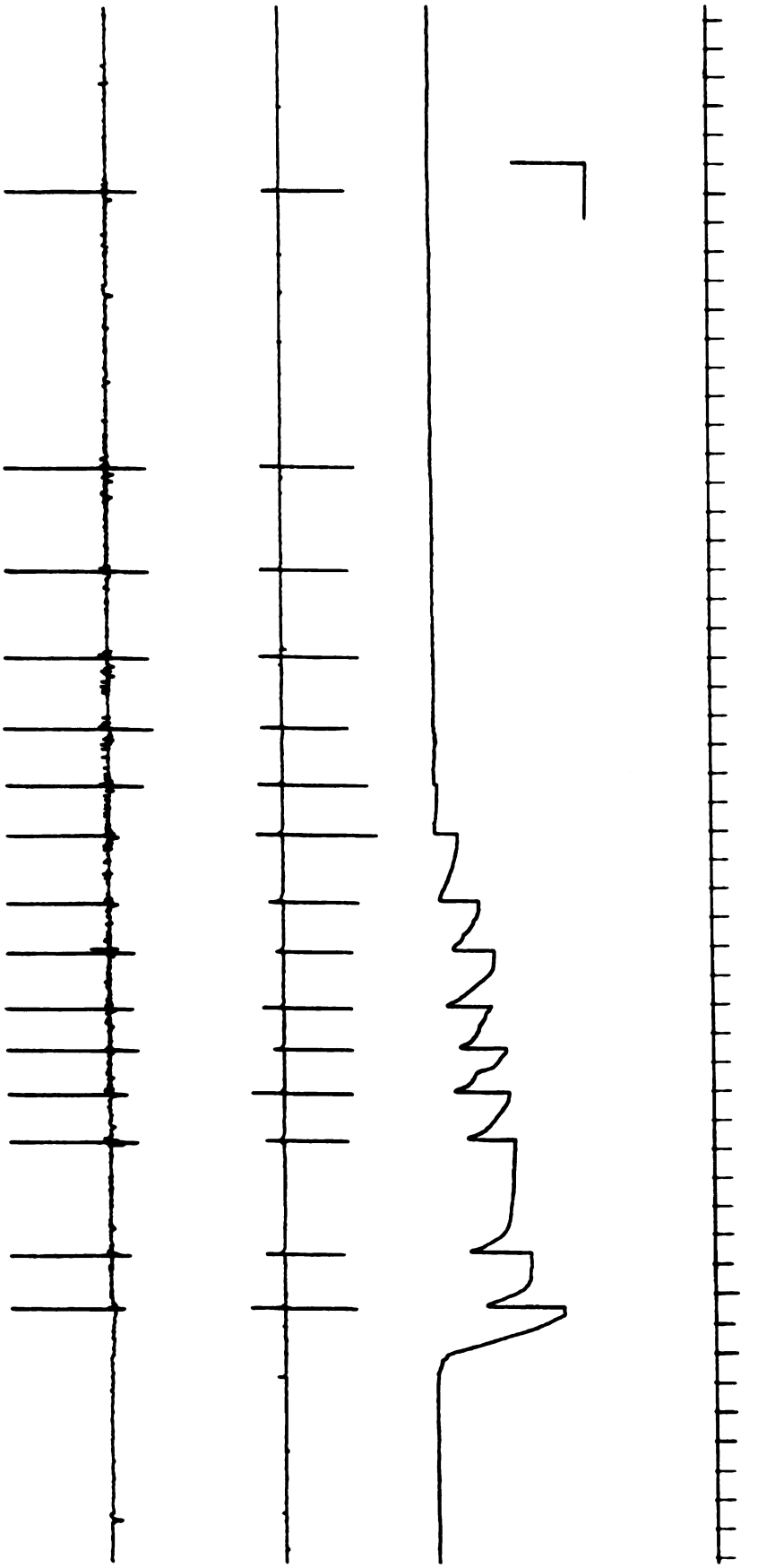

Figure 2.

The maximum contraction frequency usually occurred within the first hour following the onset of an active period. Succeeding changes in frequency were dependent on the duration of the active period. If the active period was relatively short-lived (1-3 hours), the frequency of rhythmic contractions was maintained at a fairly constant level throughout the active period. Figure 2 is an example of this. In those animals which had longer lasting active periods (more than 3 hours), a maximum frequency of contractions was reached shortly after the commencement of the period but the frequency then decreased to a level which was maintained for the remainder of the active period. This sequence of events is shown in figure 3. Occasionally, a slight increase in rhythmic frequency occurred just prior to the termination of the active period and entrance into the rest period.

The rhythmic contractions differed as to the rates of relaxation following each contraction. The changes in rate of relaxation were correlated with the point during the active period at which the contraction occurred. At the initiation of the active period, the contractions were followed by quick relaxations and valve separation reached the pre-contraction level prior to each subsequent contraction. Thus, the valve separation was relatively constant over most of the active period except for the transitory increases in tension due to each rhythmic contraction.

At the transition from an active period back to a rest period, there was a decrease in the rate of relaxation following each contraction so that each subsequent contraction left the valves a bit closer together than they had been prior to the contraction. The

Figure 3. Relationship between valve separation and frequency of rhythmic contractions at the initiation of an active period. The maximum frequency of rhythmic contractions normally occurred shortly after the initiation of the active period at the point of maximum valve separation. The frequency then decreased to a low level which was maintained throughout the active period.

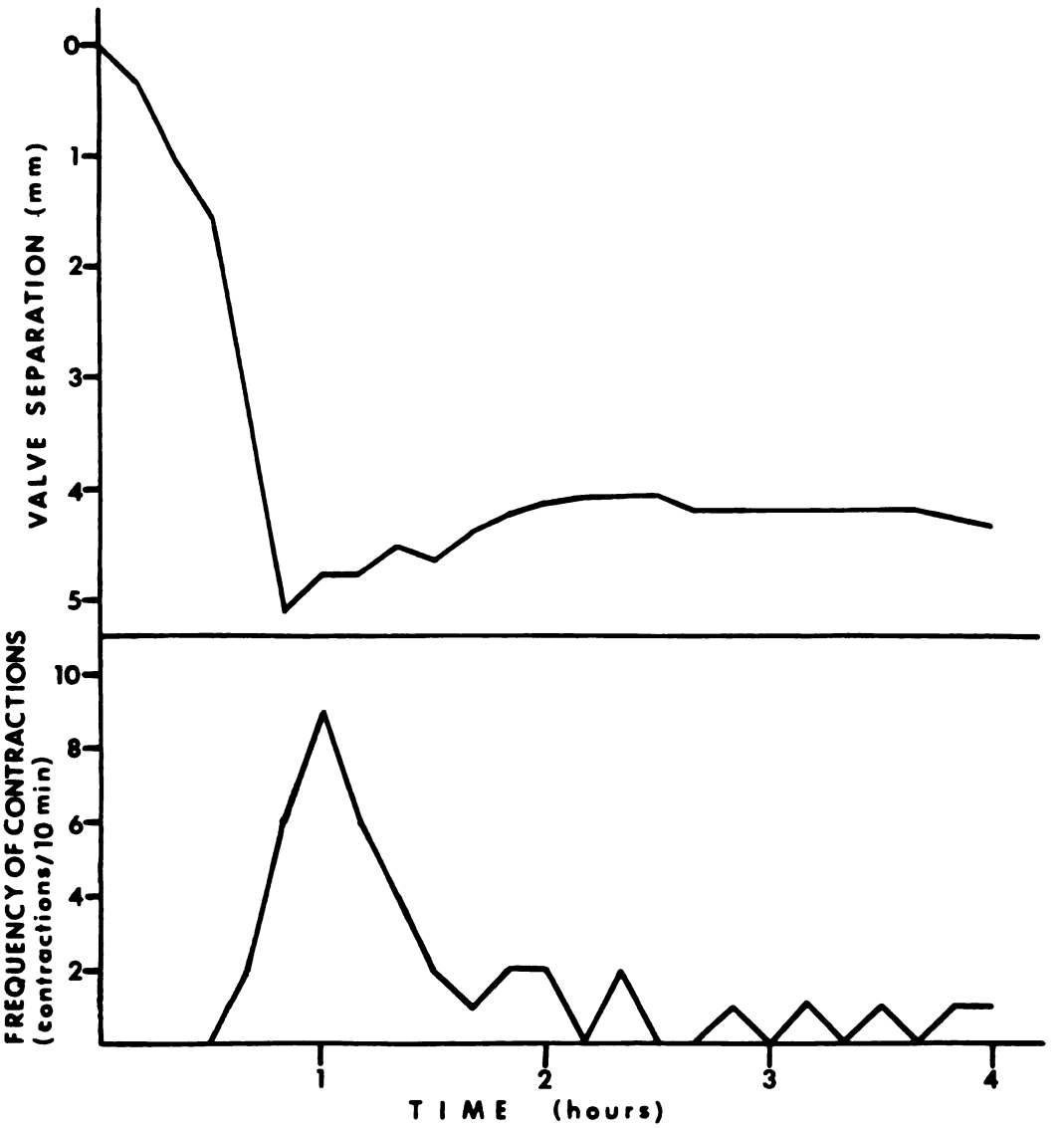
VALVE SEPARATION (mm)
0
1
2
3
4
5
FREQUENCY OF CONTRACTIONS
(contractions/10 min)
10
8
6
4
2
1
2
3
4
TIME (hours)

Figure 3.

result was that the valves closed in a stepwise manner corresponding to the series of rhythmic contractions, each accompanied by an increase in tension which was maintained until a subsequent contraction increased the tension even further. The decrease in rate of relaxation following each rhythmic contraction at the termination of an active period is shown in figure 4. Rates of relaxation can be compared to the valve separations over the same time period. All of the parameters measured showed extreme variability between animals as well as among active-rest cycles in individual animals.

Figure 4. Relationship between valve separation and rate of relaxation following rhythmic contractions at the termination of an active period. The decrease in valve separation is brought about by a decrease in the rate of relaxation following each rhythmic contraction. The displacements due to each contraction thus summate and the valves close in a stepwise manner corresponding to the rhythmic contractions.

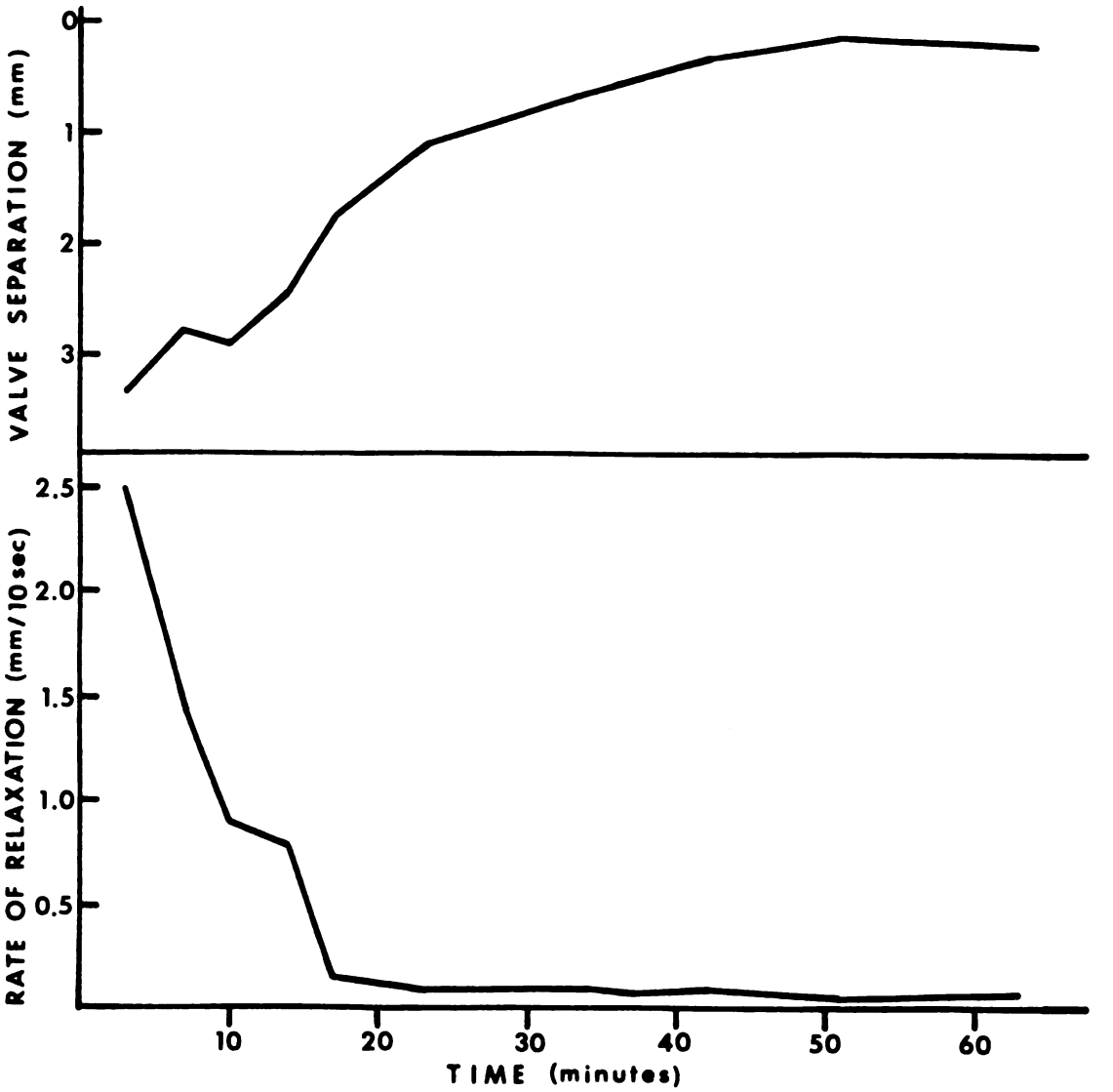
VALVE SEPARATION (mm)
0
1
2
3
RATE OF RELAXATION (mm/10 sec)
2.5
2.0
1.5
1.0
0.5
10
20
30
40
50
60
TIME (minutes)

Figure 4.

5HT Experiments

5-hydroxytryptamine (10^{-7} M. to 10^{-5} M.), when injected into either the anterior or posterior adductor muscle, was effective in causing nearly immediate relaxation of the adductors as evidenced by an increase in the separation of the valves. When the drug was administered during a rest period, the result was the initiation of an active period as is shown in figure 5. The increase in valve separation was accompanied by an initiation of rhythmic contractions. The rates of relaxation following the contractions soon reached a maximum, at the point of maximal valve separation, and then gradually decreased as the valves reclosed. Injection of higher concentrations of 5HT (10^{-5} M.) caused slightly greater valve separations and greater rates of relaxation following the rhythmic contractions as is shown in figure 5B.

When 5HT was administered to either the anterior or posterior adductor during an active period, the valve separation became greater than that recorded during spontaneous active periods. Similarly, the frequency of rhythmic contractions increased in many of the animals, especially at higher concentrations of 5HT, and the rates of relaxation following the rhythmic contractions also increased to a higher rate than that recorded during spontaneous active periods. These effects at concentrations of 10^{-7} M. and 10^{-5} M. 5HT are shown in figure 6. All effects were more pronounced and longer lasting at the higher concentration.

Injection of 5HT into either the anterior or posterior adductor proved to be sufficient to cause an immediate increase in valve

Figure 5. Effect of 5HT on a mussel during a rest period. 5HT was injected into the posterior adductor at the arrows. Concentrations of 5HT were 10^{-7} M. in A and 10^{-5} M. in B. At both concentrations, injection of 5HT caused an increase in valve separation and initiation of rhythmic contractions resembling those accompanying the normal initiation of an active period. Vertical calibration: 1.0 mm. Horizontal calibration: 1 min.

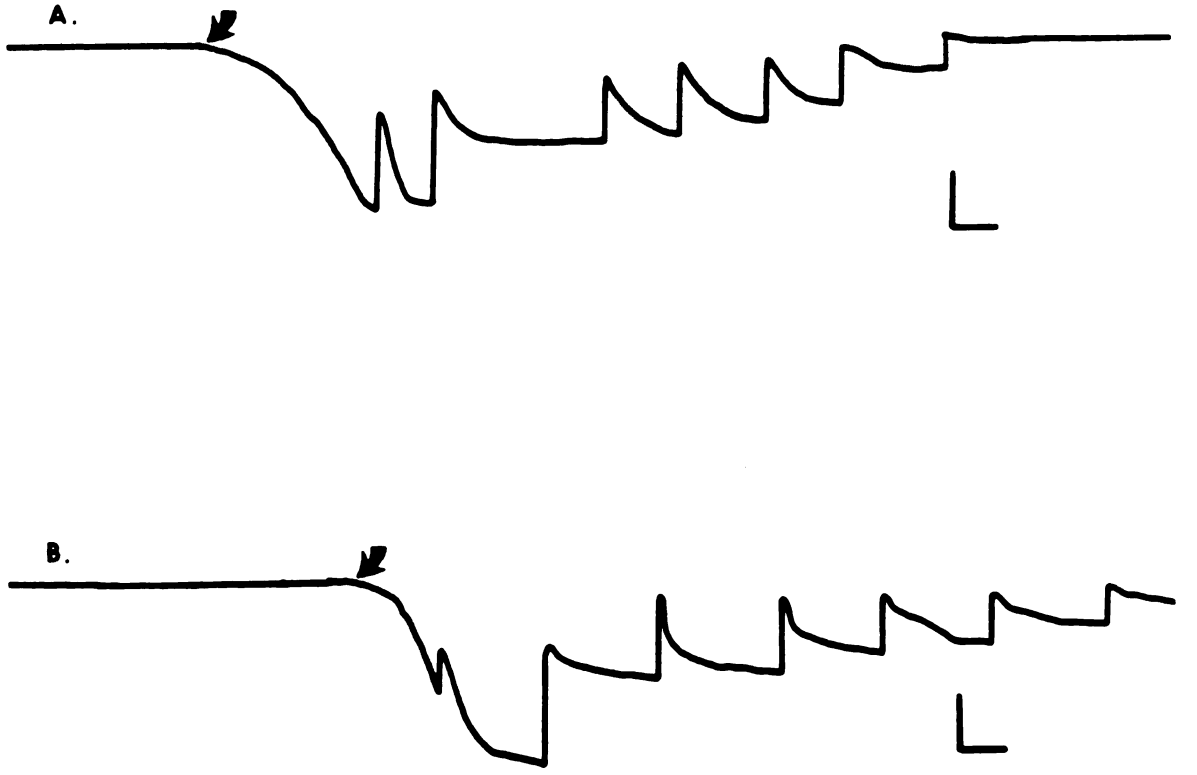

Figure 5.

Figure 6. Effect of 5HT on a mussel during an active period. 5HT was injected into the posterior adductor muscle at the arrows. Concentrations of 5HT were 10^{-7} M. in A and 10^{-5} M. in B. Injection of 5HT during an active period caused an increase in valve separation and an increase in the rate of relaxation following rhythmic contractions which often exceeded those recorded in control animals during an active period. Vertical calibration: 1.0 mm. Horizontal calibration: 1 min.

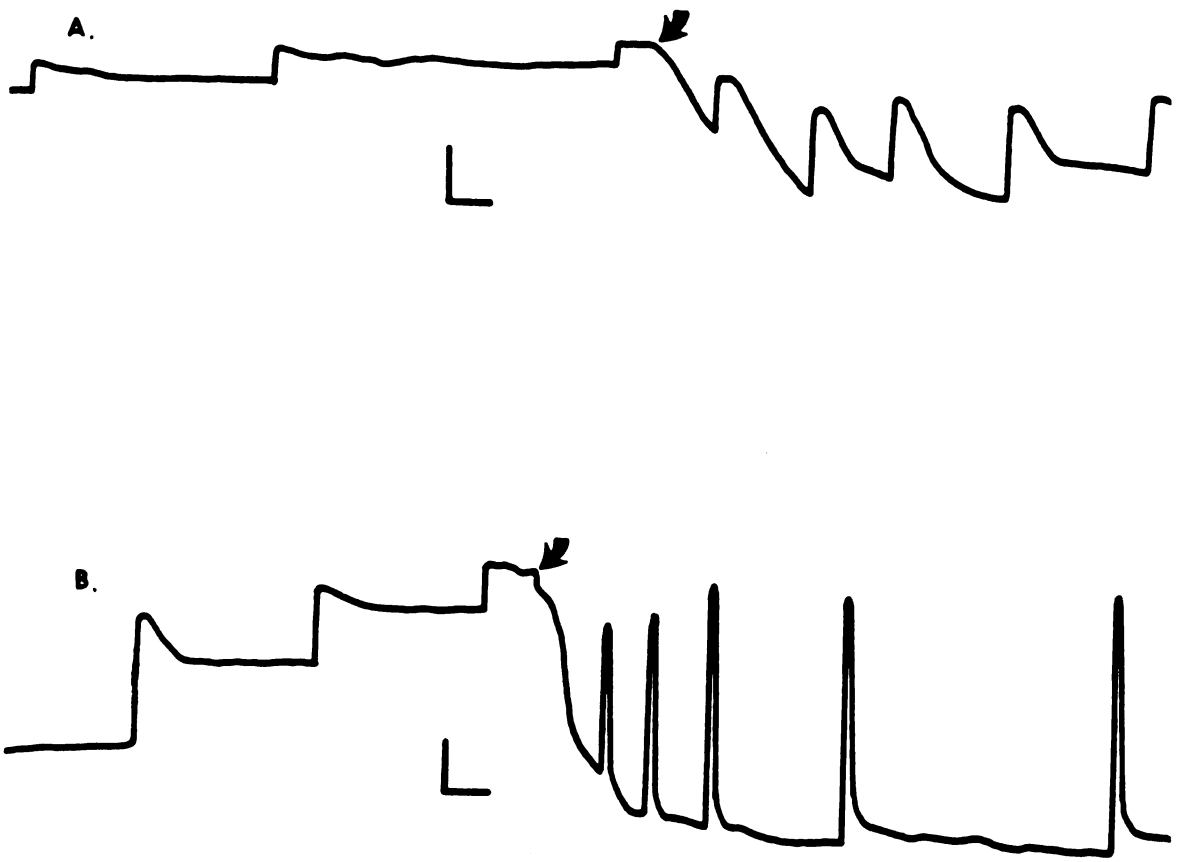

Figure 6.

separation during an active period. Since adduction of the valves normally results from synchronous contractions of both anterior and posterior adductors, an abduction would require a simultaneous release of tension in both adductors. The possibility that the 5HT was reaching the uninjected muscle by diffusing through the mantle cavity was tested by injecting similar concentrations of 5HT directly into the mantle cavity and comparing the results with injections into either adductor. Figure 7 compares the effects of injecting 5HT into the anterior adductor, the posterior adductor or the mantle cavity. Change in valve separation was measured as the difference between the amount of separation just prior to the injection and the maximum separation following the injection. Changes due to injection into the mantle cavity were not significant even when 10^{-5} M. 5HT was injected (t-test). The difference in valve separation as a result of injection of 5HT into the anterior adductor vs. the mantle cavity and the posterior adductor vs. the mantle cavity were statistically significant ($p < .05$) at all concentrations of 5HT. Differences due to injection of the drug into the anterior vs. the posterior adductor were not significant.

The effects of 5HT on the rates of relaxation following rhythmic contractions are shown in figure 8. 5HT injection into the mantle cavity did not have a significant effect on relaxation rate at any of the concentrations tested. Injection of 5HT at concentrations of 10^{-6} M. or higher into the anterior adductor significantly increased relaxation rate ($p < .05$) while injection into the posterior adductor caused a significant change at 10^{-5} M. ($p < .05$). Differences in

Figure 7. Changes in valve separation following the injection of 5HT into the anterior adductor, posterior adductor and mantle cavity. Each point represents the mean of the responses of at least 5 animals. Vertical bars represent $\pm$ one standard error. Change in valve separation was measured as the difference between the pre-injection separation and the maximum separation following injection. mc: mantle cavity; pa: posterior adductor; aa: anterior adductor.

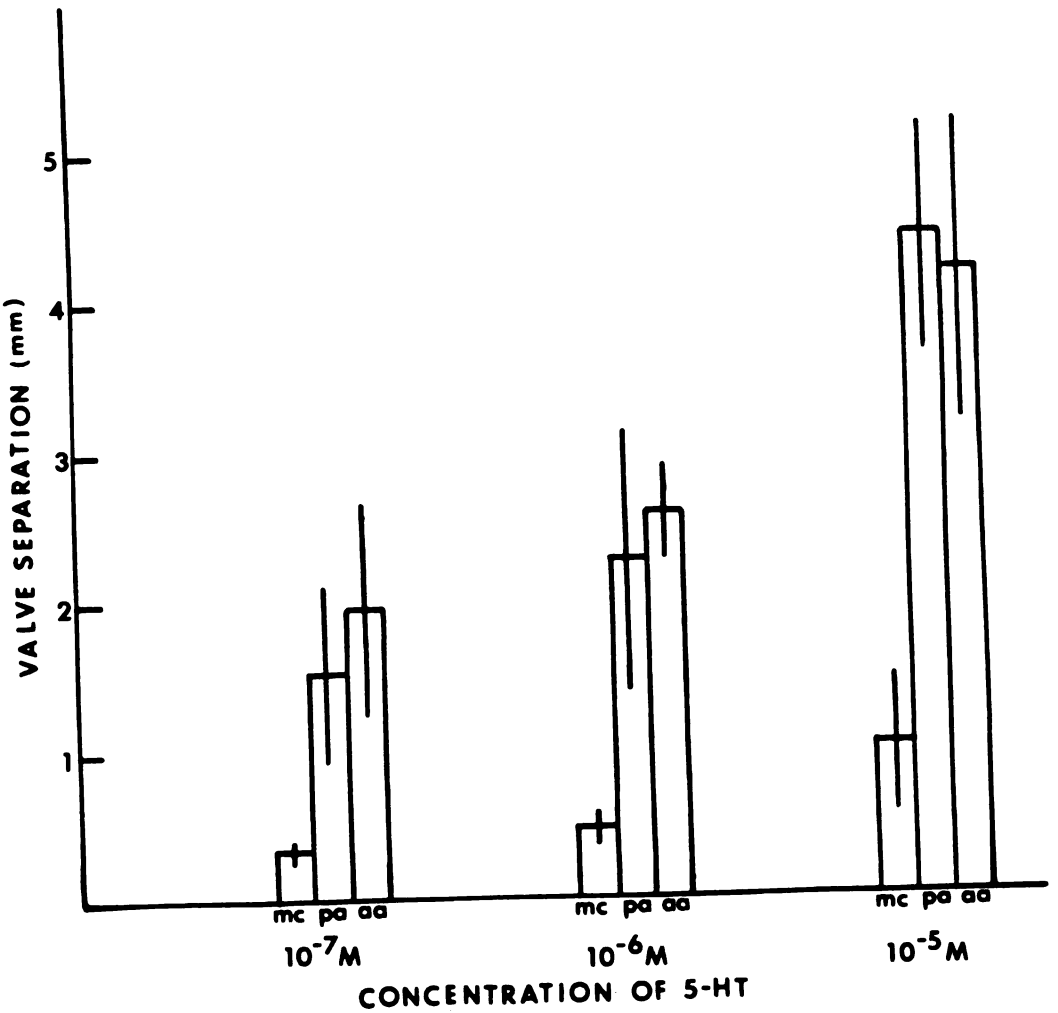
VALVE SEPARATION (mm)
5
4
3
2
1
mc pa aa
mc pa aa
mc pa aa
10-7M
10-6M
10-5M
CONCENTRATION OF 5-HT

Figure 7.

Figure 8. Changes in rate of relaxation following rhythmic contractions following the injection of 5HT into the anterior adductor, posterior adductor and mantle cavity. Each point represents the mean of the responses of at least 5 animals. Vertical bars represent $\pm$ one standard error. Rate of relaxation was measured as the difference between the pre-injection rate of relaxation and the maximum rate of relaxation following injection.
mc: mantle cavity; pa: posterior adductor;
aa: anterior adductor.

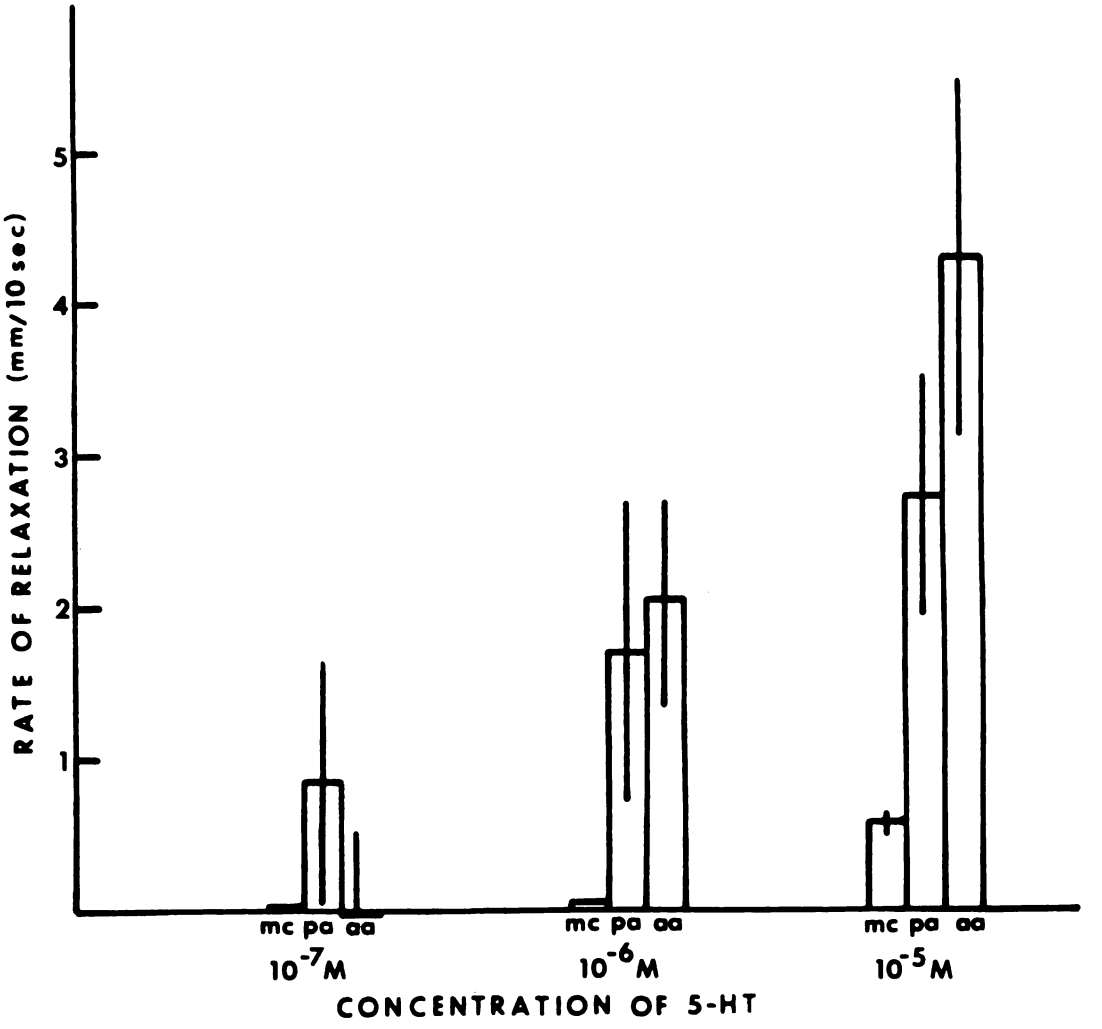
RATE OF RELAXATION (mm/10 sec)
5
4
3
2
1
mc pa aa
mc pa aa
mc pa aa
10-7M
10-6M
10-5M
CONCENTRATION OF 5-HT

Figure 8.

rate of relaxation between anterior vs. posterior injections were not significant at any concentration.

Figure 9 compares the durations of the 5HT effect when the drug is injected into the anterior or posterior adductor during an active period. Duration was measured as the time required for the rate of relaxation following rhythmic contractions to return to pre-injection levels. This time was usually identical to the time required for the valve separation to return to pre-injection levels. Posterior injections tended to cause longer lasting effects although the differences between anterior and posterior injections were not statistically significant.

Thus the changes brought about by injection of 5HT into either adductor during a rest period closely resembled those occurring in the normal initiation of an active period. When 5HT was injected during an active period, there was an increase in valve separation and in the rates of relaxation following rhythmic contractions and often in the frequency of rhythmic contractions. All effects tended to be more extreme at higher concentrations of 5HT. Neither injection of 5HT into the mantle cavity nor injection of saline into either adductor resulted in a significant alteration of any of these parameters.

Figure 9. Duration of the 5HT effect. Each point represents the mean of the responses of at least 5 animals. Vertical bars represent $\pm$ one standard error. Duration was measured as the time required for the rates of relaxation following the rhythmic contractions to return to pre-injection levels.
aa: anterior adductor; pa: posterior adductor.

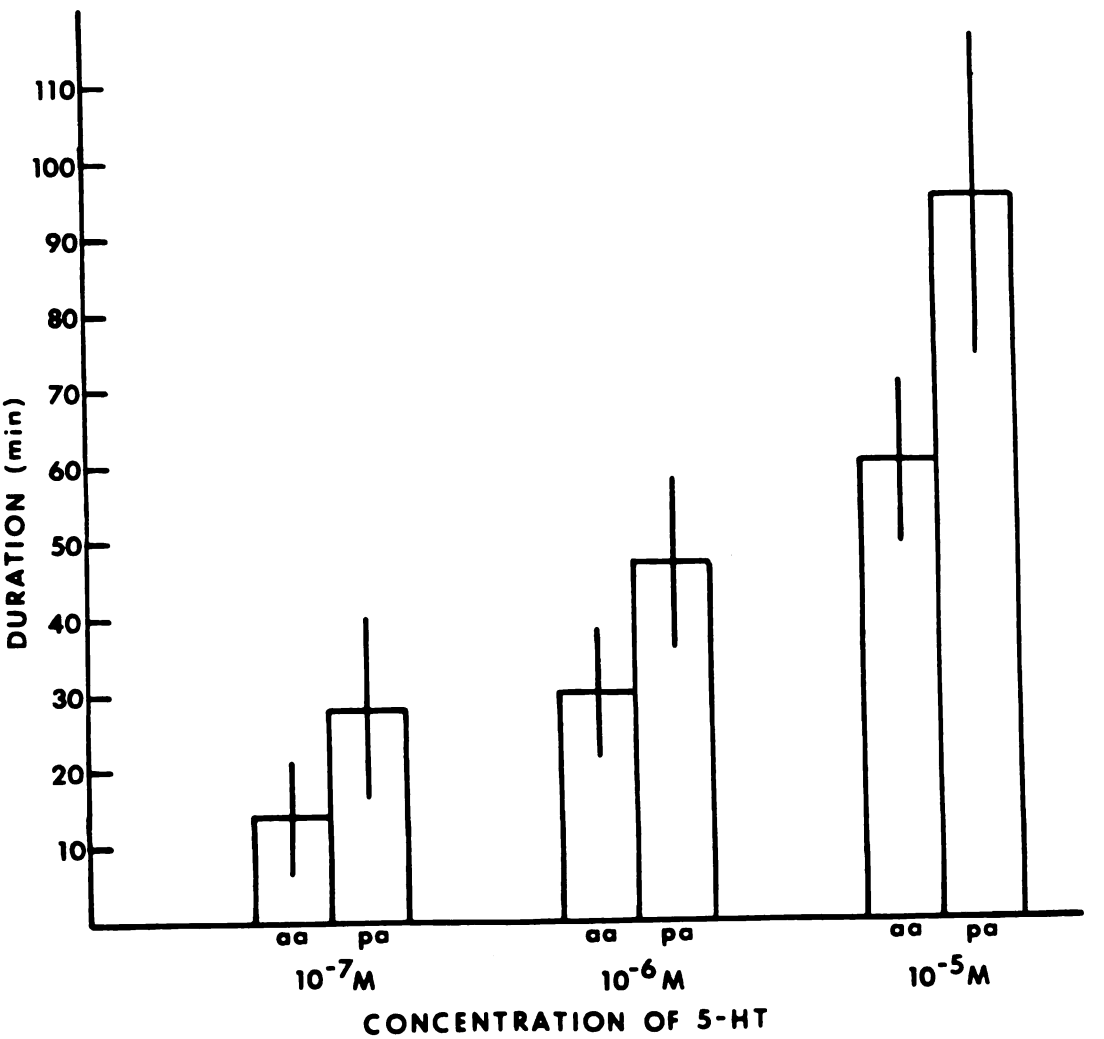
110
100
90
80
70
60
50
40
30
20
10
DURATION (min)
aa
pa
aa
pa
aa
pa
10-7M
10-6M
10-5M
CONCENTRATION OF 5-HT

Figure 9.

Isolated Muscle Experiments

The rate of relaxation of an isolated whole muscle or isolated muscle-visceral ganglion preparation under an applied load was calculated as a measure of catch in these preparations. Both preparations exhibited a considerable stretch resistance to an applied load of 38 grams. The rate of relaxation of the isolated muscle was about 5 times higher, under these conditions, than that of the muscle-ganglion preparation as is shown in figure 10, controls (significant at $p < .05$).

Spontaneous rhythmic contractions were never observed in isolated muscle or muscle-ganglion preparations. Electrical stimulation with a.c. current (0.5-500 msec. duration, 5-60 volts, 0.2-100 pulses per second) failed to elicit a contraction although this method was shown to be effective in work on the ABRM of Mytilus (Twarog, 1967). A.c. stimulation was shown, by the same author, to increase the relaxation rate following contraction in the ABRM-pedal ganglion preparations but not in isolated ABRM preparations. In the present study, a.c. stimulation had no significant effect on the rate of relaxation of either the isolated adductor or the muscle-ganglion preparations.

Stimulation of the isolated muscle with d.c. current (15-60 volts, 10-60 seconds) resulted in an increase in tension for the duration of the stimulus. Following termination of the stimulus, the muscle slowly relaxed but the rate of relaxation was much lower than that recorded prior to stimulation. Figure 11 shows the response of an isolated muscle preparation to a d.c. stimulus (15-30-60 volts, 10 seconds). The maximum tension attained was proportional to the

Figure 10. Rates of relaxation of isolated whole muscle and muscle-ganglion preparations following injection of 5HT. Each point represents the mean of the responses of at least 5 preparations. Vertical bars represent $\pm$ one standard error. Control measurements were made prior to the injection of 5HT. im: isolated muscle preparation; dc: d.c. stimulated isolated muscle; mg: muscle-ganglion preparation.

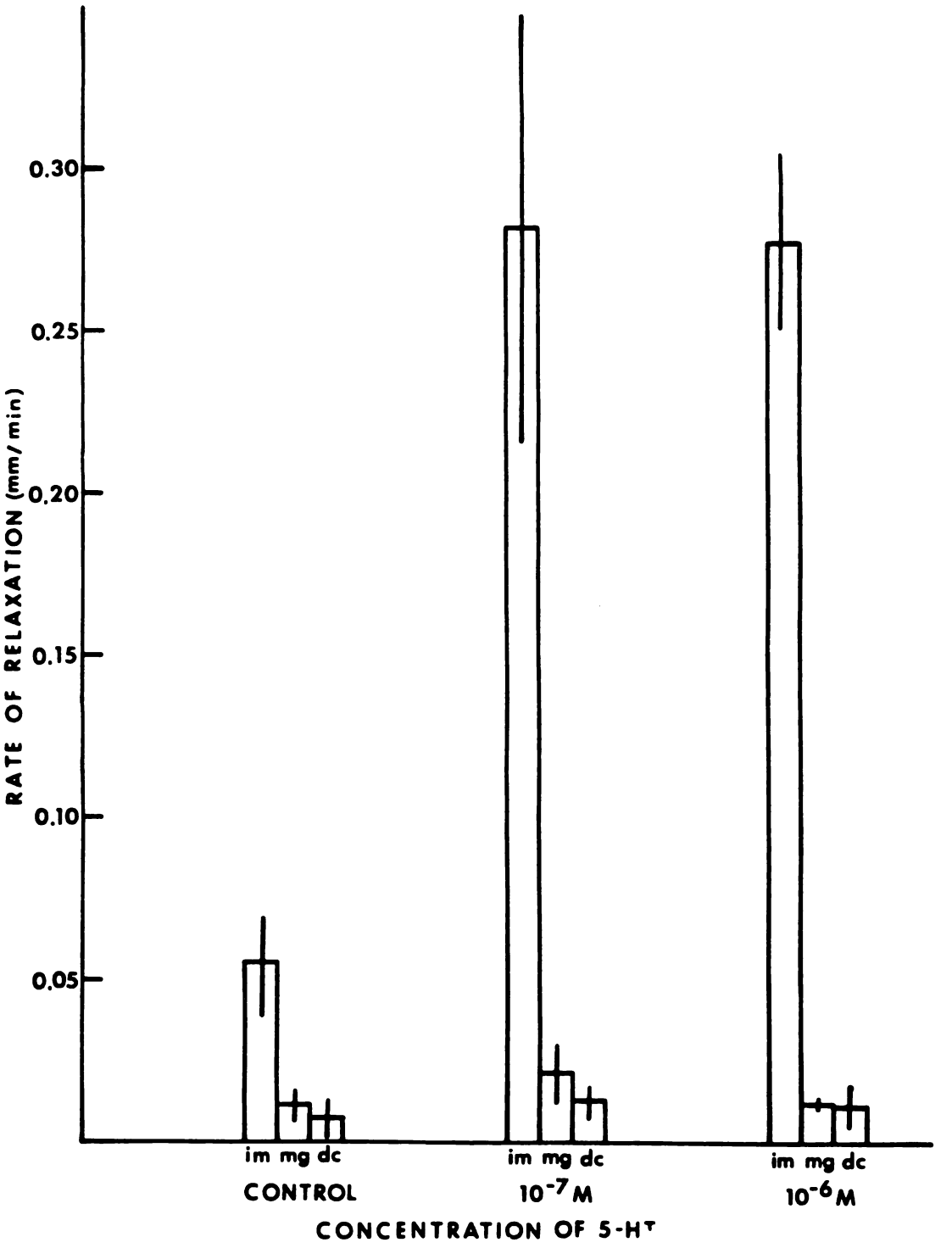
0.30
0.25
0.20
0.15
0.10
0.05
RATE OF RELAXATION (mm/min)
im mg dc
CONTROL
im mg dc
10-7M
im mg dc
10-6M
CONCENTRATION OF 5-HT

Figure 10.

Figure 11. Mechanical response of isolated adductor muscle to d.c. stimulation. The increase in tension caused by d.c. stimulation is proportional to the voltage of the applied stimulus.
Vertical calibration: 0.5 mm.
Horizontal calibration: 2 minutes.

Figure 12. Mechanical response of isolated adductor muscle to injection of 5HT. 5HT (10^{-7} M.) was injected into the muscle at the arrow resulting in an immediate increase in the rate of relaxation.
Vertical calibration: 0.5 mm.
Horizontal calibration: 2 minutes.

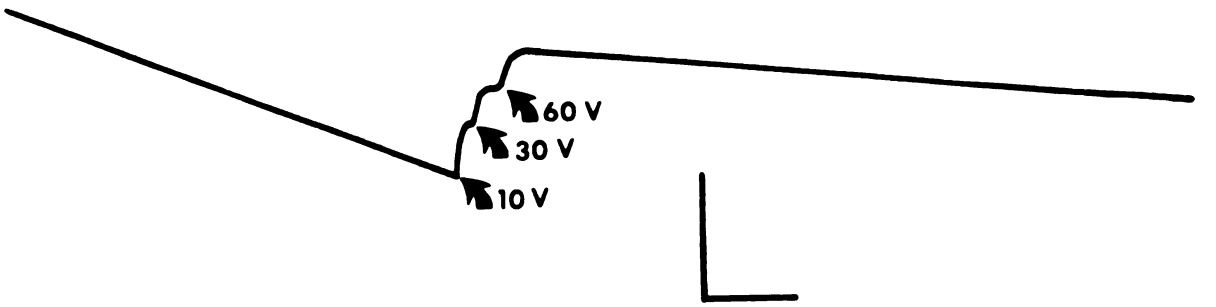

Figure 11.

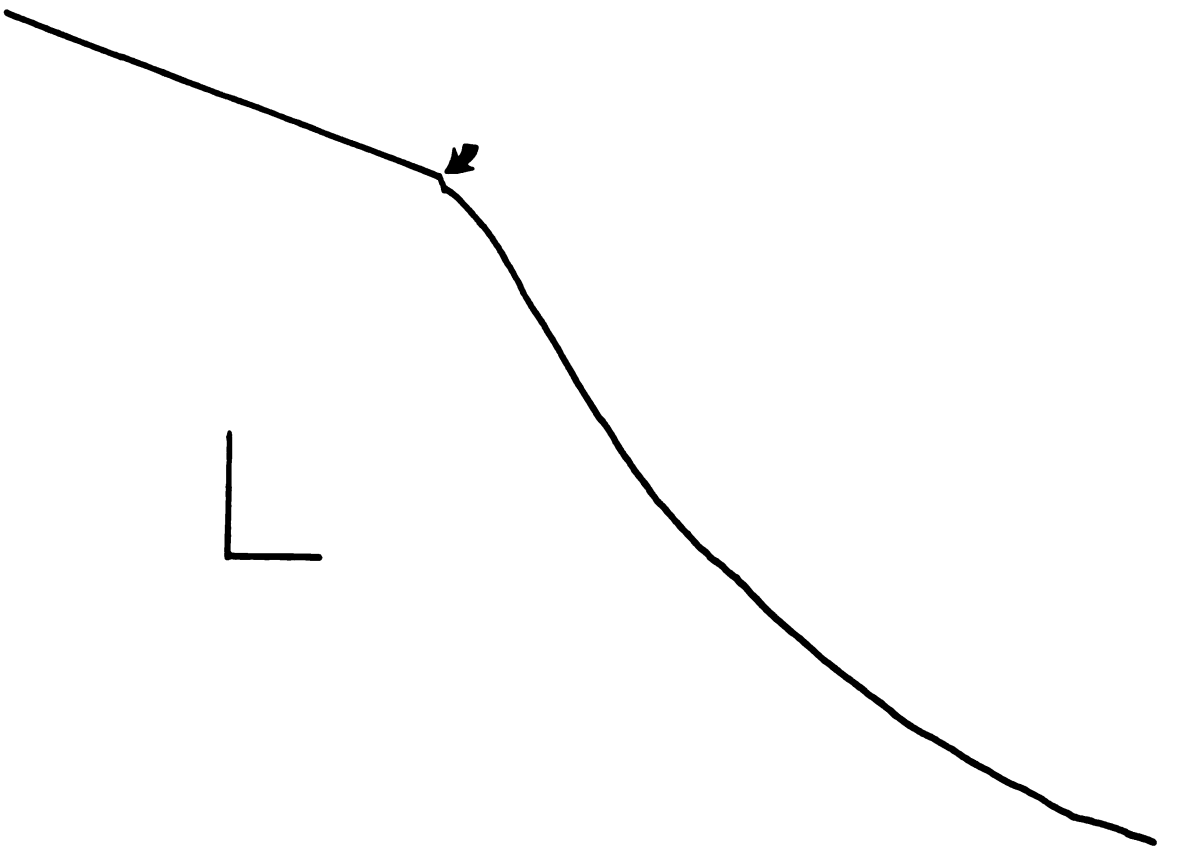

Figure 12.

voltage of the applied stimulus as is shown in the figure. The decreased rate of relaxation following d.c. stimulation was maintained for extended periods of time.

Figure 10, controls, compares the mean rate of relaxation of the unstimulated isolated muscle with the isolated muscle which had been stimulated with d.c. current (30 volts, 60 seconds). The unstimulated muscle relaxed at a rate approximately seven and a half times higher than the d.c. stimulated muscle (significant at $p < .01$).

5HT (10^{-7} M.) effectively increased the rate of relaxation in the isolated whole muscle as is shown in figure 10 and figure 12, with the rate increasing by more than five times following the injection of the drug (significant at $p < .01$). However, 5HT did not have a significant effect on relaxation rate when injected into either the muscle-ganglion preparation or the d.c. stimulated isolated muscle (figure 10). Higher concentrations of 5HT (10^{-6} M.) did not significantly further increase the relaxation rate. The relaxation rates of muscle-ganglion preparations and d.c. stimulated muscles remained unaffected in concentrations of 5HT up to 10^{-3} M.

Muscle Bundle Experiments

A final series of experiments was conducted on isolated muscle bundles taken from the posterior adductor muscle. This assured the exclusion of any extraneous neural factors possible affecting the maintenance of catch as well as eliminating the effect of direct injection of 5HT into the muscle by allowing the administration of the drug by diffusion from the medium. In addition, this preparation allowed the concentration of 5HT to be maintained by preventing the diffusion of the drug out of the muscle and the subsequent dilution in the medium inherent in the previous experiments.

Isolated muscle bundles are capable of maintaining a considerable resistance to stretch as evidenced by the slow rate of relaxation occurring under an applied load of 25 grams. 5HT (10^{-7} M.) significantly increased this rate ($p < .005$) as is shown in figure 13. Relaxation rates were measured 3 minutes after the 5HT was administered to allow sufficient time for the drug to diffuse into the muscle bundle. Higher concentrations of 5HT (10^{-6} M.) did not significantly further increase the maximum rate attained with 10^{-7} M. 5HT (figure 13).

Figure 13. Rate of relaxation of isolated muscle bundle following injection of 5HT. Each point represents the mean response of 5 different isolated muscle bundles. Vertical bars represent $\pm$ one standard error. Control refers to the rate of relaxation of the muscle bundle prior to the injection of 5HT.

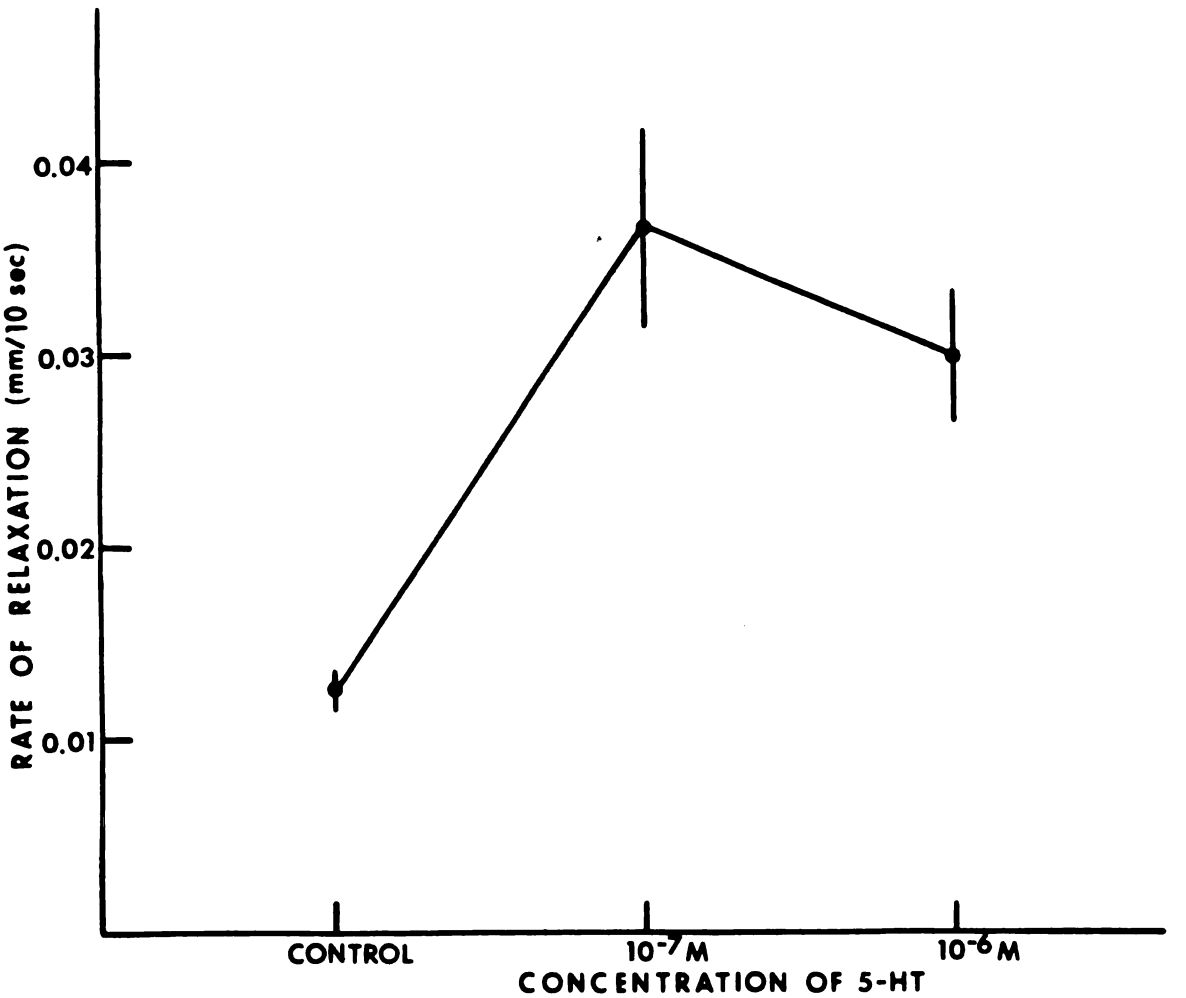
RATE OF RELAXATION (mm/10 sec)
0.04
0.03
0.02
0.01
CONTROL
10⁻⁷M
10⁻⁶M
CONCENTRATION OF 5-HT

Figure 13.

DISCUSSION

The species examined in this study exhibit periodic and rhythmic activities similar to those of Anodonta cygnea (Marceau, 1909, Barnes, 1955, Salanki, 1970), Mytilus edulis (Lowy, 1953) and Crassostrea angulata (Millman, 1964). The periodicity consists of alternating periods of activity and rest as demonstrated by the degree of tonus (catch) in the adductor muscles. Rest is that state in which tonus is extremely high and the valves are tightly closed. The active period is characterized by reduced adductor tonus and a variable separation of the valves.

Rhythmic Activity

Rhythmic activity during an active period consists of a series of sub-maximal valve adductions and associated bursts of electrical activity which are recorded simultaneously from both the anterior and posterior adductor muscles. The frequency of the rhythmic contractions appears to be related to the periodicity in that the highest rhythmic frequencies predictably occur at the initiation and termination of each active period. Valve adductions are not recorded during a rest period since the valves are already tightly closed. The electrical activity, however, usually continues at a decreased frequency well into the rest period and provides a means for measuring rhythmic activity during rest.

The rhythmic contractions differ as to the rates of relaxation following each contraction through the course of the active period. Shortly after the onset of the period, the relaxation rates are quite high so that each contraction results in a short-lived valve displacement immediately followed by a return of the valve positions to the pre-contraction level. The relaxation rates become increasingly lower as the active period proceeds until, at the termination of the period, very little relaxation occurs after each rhythmic contraction. Successive contractions, then, act to increase the overall tonicity of the adductors in a stepwise manner and, hence, result in the initiation of a rest period. The rhythmic contractions appear to be essential in establishing the increase in tonus which preceeds the subsequent rest period. At no time does the tonus significantly increase in the absence of rhythmic contractions. The rhythmic contractions thus are phasic in the early stage of the active period but become increasingly tonic as the active period proceeds.

Effect of 5HT

Periodicity

5HT, when injected into either adductor muscle, reduces the level of tonus already established in the muscle and simultaneously reduces the ability of the muscle to maintain tonus following a rhythmic contraction. When 5HT is administered during a rest period, the increase in valve separation due to the reduction in adductor tonus is accompanied by an initiation of rhythmic contractions. The effect of 5HT injection thus resembles the normal transition from a rest to an active period.

In control animals, the adductor tonus, and thus the valve

separation, is apparently controlled by the concentration of 5HT in the muscles. Normally there is a very low level of 5HT in the adductors during rest (Salanki and Hiripi, 1970, Salanki et. al., 1974). This could account for the extremely high muscle tonus and closure of the valves. Increase of 5HT level decreases the muscle tonus, resulting in a separation of the valves and thus initiating an active period. There is a relative consistancy in the maximum valve separations during successive active periods in individual control animals. Presumably, the amount of 5HT released in the adductors is also relatively constant among the active periods in an individual animal.

The response to injection of 5HT during an active period indicates that the muscle maintains a considerable tonus even when the valves are separated. The maximum valve separation during an active period can be increased by injection of higher concentrations of 5HT than those normally found during an active period. The valve separation thus increases with the addition of 5HT to a greater distance than that which is normally maintained during an active period.

This effect points out a confusion in the definition of catch. The adductor muscles are usually described as being in catch only during a rest period when the valves are tightly closed. Initiation of an active period would then involve the release of catch. However if the level of adductor tonus, which determines the valve separation, is continuously variable and dependent on the concentration of 5HT, then the description of an adductor as either being in catch or not, would be an oversimplification of the variable tonus of the muscles.

Rhythmic Contractions

The effect of 5HT on the rates of relaxation following rhythmic contractions appears to be consistent with the effect on muscle tonus. Consecutive active periods, in control animals, contain rhythmic contractions which have relaxation rates which are dependent on both the degree of valve separation and the amount of time the animal has been in an active period. Both of these parameters are, in turn, dependent on the 5HT level in the adductors. Relaxation rates tend to be quite high at the initiation of the active period when 5HT levels are assumed to be at their maximum. As the active period proceeds, there is a corresponding decrease in relaxation rate, presumably due to a decrease in 5HT concentration, which eventually results in a decrease in valve separation. Injection of higher concentrations of 5HT increases both the valve separation and the relaxation rates as would be expected.

Synchrony of the Adductor Muscles

The rhythmic contractions and the development of muscle tonus occur simultaneously in both the anterior and posterior adductors. Thus, in order to initiate an increase in valve separation, simultaneous decreases in tonus must occur in both muscles. The present results indicate that injection of 5HT into either adductor muscle apparently affects the uninjected as well as the injected muscle. However, injection of 5HT into the mantle cavity where it is free to diffuse toward both adductors is ineffective in causing a significant increase in valve separation. The synchrony between the two adductors, upon injection of 5HT, is, thus, apparently accomplished through neural circuits,

probably by way of the cerebro-visceral connective rather than by diffusion. The details of this mechanism have yet to be worked out although electrical recordings have been made from the cerebro-visceral connective which show that there is a burst of electrical activity in this connective which corresponds to the rhythmic contractions (Salanki and Varanka, 1972). A mechanism of transport of 5HT by way of the cerebro-visceral connective has been proposed (Salanki and Hiripi, 1970).

Isolated Muscle Studies

With the exception of Millman (1964), the data presented on isolated posterior adductor muscles, isolated muscle bundles and adductor-visceral ganglion preparations are the only such data available. It is evident that 5HT acts on the adductor muscle directly rather than by way of the visceral ganglion or other components of the central nervous system. It is likely that the rhythmic contractions, on the other hand, are centrally determined as proposed by Salanki and Varanka (1972). Thus all rhythmic contractions were abolished in the isolated adductor muscle yet 5HT retained its usual effect on muscle tonicity. In the present study, rhythmic contractions were also absent from the muscle-visceral ganglion preparations. It is likely that the initiation of rhythmic contractions originates within some higher portion of the central nervous system, the cerebral ganglion for instance. In support of this idea, 5HT is apparently capable of affecting rhythmic contractions when applied to the cerebral ganglion (Salanki, 1963) presumably through transport to the muscle.

It should be noted that Millman (1964) reported some rhythmic

contractions in the adductor- visceral ganglion preparation. This is a discrepancy as compared with present results which has not been resolved. Salanki and Varanka (1972) reported rhythmic bursting activity in isolated visceral ganglia and, in a short communication (Salanki and Zs Nagy, 1970), reported some continued rhythmic contractions even when all ganglia had been removed. The authors attributed this latter anomaly to incomplete ablation of neural elements since the effect was found in a very small percentage of the subjects. In the former case, the visceral ganglia were dissected along with lengths of the pallial nerves and the paired cerebro-visceral connectives. It is not clear whether the bursting, as recorded from the pallial nerves and cerebro-visceral connectives is directly associated with the rhythmic contractions.

Several authors have reported a large increase in the tonicity of the posterior adductor with the cutting of the cerebro-visceral connectives (Lowy, 1953, Puppi, 1963) or with denervation and removal of the muscle (Millman, 1964). Millman (1964) further reported that, when the visceral ganglion was left attached to the isolated muscle, the muscle fatigued and decreased tonicity less rapidly than when the ganglion was removed. The present study confirms these effects. In addition, 5HT was found to be effective in releasing catch and decreasing resistance to stretch in all isolated whole muscle and muscle bundle preparations but was ineffective in causing similar changes in the whole muscle-visceral ganglion preparation. This result again differs from Millman's data (1964) but Millman's muscle-ganglion preparation consisted of a small muscle bundle with the visceral ganglion attached. Since the innervation of the adductor by the visceral ganglion is extensive,

it is quite difficult to dissect a small bundle of fibers without also damaging the visceral ganglion. It is conceivable that Millman could have caused such damage to the ganglion or the innervation to the muscle resulting in an alteration of the visceral ganglion's influence on the maintenance of tonicity. From the present results, it appears that the visceral ganglion may have an excitatory influence on the posterior adductor muscle which is capable of maintaining catch even in the presence of 5HT at concentrations which would normally cause the release of the maintained tension. It is not known whether this effect is important in the intact animal.

The effect of 5HT on d.c. stimulated, isolated whole muscle presents another puzzle. D.c. stimulation has been believed to initiate a normal catch contraction and the resistance to stretch and rate of relaxation following the contraction resemble the spontaneous catch initiation. Yet 5HT has no immediate effect on releasing this tension and increasing the rate of relaxation. There are, apparently, differences between the tension developed in the d.c. initiated contraction as compared to the muscle which has spontaneously entered into catch. The spontaneous catch is developed through a series of rhythmic contractions each of which has a low rate of relaxation so that catch is developed in a stepwise manner. Since rhythmic contractions have been eliminated in the isolated muscle, the tension development in the d.c. stimulated muscle takes the form of a slow development of tension which is proportional to the voltage and duration of the applied stimulation. Quite different mechanisms may be involved in the two types of contractions.

SUMMARY

1. Active-rest periodicities and rhythmic frequencies of freshwater mussels are described. Active periods are characterized by a separation of the valves and a series of rhythmic contractions each of which is accompanied by bursts of electrical activity in both adductor muscles.
2. Injection of 5HT into either adductor muscle during a rest period causes a valve abduction and initiation of rhythmic contractions identical to those accompanying the initiation of an active period in control animals.
3. When 5HT is injected into either adductor during an active period, there is an increase in valve separation and an increase in the rate of relaxation following each rhythmic contraction. The result is a larger valve separation and a higher relaxation rate than those recorded in control animals during an active period.
4. 5HT causes an increase in the rate of relaxation under a constant load when the drug is administered to an isolated posterior adductor muscle or an isolated bundle of fibers obtained from this muscle. Thus 5HT acts on the muscle itself rather than by way of the CNS or other neural elements.

5. 5HT has no significant effect on the relaxation rate of posterior adductor muscle-visceral ganglion preparations indicating that there is an excitatory influence on the adductor due to the presence of the visceral ganglion. The muscle-ganglion preparation is capable of maintaining tension even in the presence of 5HT.
6. D.c. stimulation causes an increase in the tension of the posterior adductor which is proportional to the voltage of the applied stimulus. This tension is maintained following the termination of the stimulus as is indicated by a slow rate of relaxation. This rate is not increased with the addition of 5HT.

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LITERATURE CITED

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