Postjunctional Potentials and Cardiac Acceleration in a Mollusc (*Dolabella auricula*)

Many investigators have studied the acceleratory effect of stimulation of cardio-regulatory nerves on heart rhythm. For example, A. J. CARLSON[1] reported that stimulation of the cardio-accelerator nerves of *Aplysia* produced rhythmical contractions in the quiescent heart. K. MATSUI[2] and A. EBARA[3] have shown that the heart of another closely related tectibranch, *Dolabella auricula*, is controlled by both excitatory and inhibitory nerves. However, there seems to be no direct evidence concerning the effect of the excitatory junctional potential (EJP) on the action potential of the myogenic molluscan heart[4,5]. The observations presented here are concerned with the mode of effect of the EJP[6] on the rhythmical action potential of the myogenic heart of *Dolabella auricula*.

Ventricular muscle of *Dolabella* beats spontaneously when stretched[7,8]. The preparation was adequately stretched and pinned on the floor of a lucite chamber filled with hemolymph. The membrane potential was recorded with an intracellular electrode inserted into a cardiac muscle cell at the ventricular region near the auriculo-ventricular valve, where the EJP was recorded as a depolarizing potential of small size. This region is densely innervated by the excitatory nerve, and shows more spontaneous activity than other regions of the ventricle. The regulatory nerve was suspended over the perfusion medium by means of the stimulating electrodes (Ag-AgCl). Care was taken that the stimulation pulse did not excite cardiac muscle directly. Other details of experimental procedure were similar to those described previously[6].

The membrane potential difference during a spontaneous cardiac cycle ranged from -7 mV to -56 mV; it thus never reached the reversal potential of -61 mV for the inhibitory junctional potential[6]. The effect of a stimulation frequency, which is higher than that of the spontaneous cardiac cycle, on the spontaneous action potential of the heart is shown in the Figure. When the frequency of stimulation was slightly higher than that of the spontaneous action potentials, an induced action potential was produced regularly by each stimulus. In Figure A, the interval between spontaneous action potentials is 4.4 sec, and that between stimuli is 3.8 sec. The rising phase of the induced action potential is very sharp and steep, and the EJP is indistinguishable from the rising phase of the spike. This is probably due to the EJP having brought the membrane potential rapidly up to the threshold level of spike initiation, so that the spike starts instantly on the peak of the EJP.

When the frequency of stimulation becomes higher, the frequency of action potentials appears to reach a limit above which action potentials cannot follow each stimulus, and some complex phenomena occur. In Figure B the interval between spontaneous action potentials is 4.4 sec and the interval between stimuli is 2.5 sec. At this frequency some EJP's did not elicit action potentials. The second and third stimuli failed to evoke an action potential but induced an EJP on the repolarizing phase and on the summit of the action potential, respectively. Failure of these stimuli to evoke the action potential

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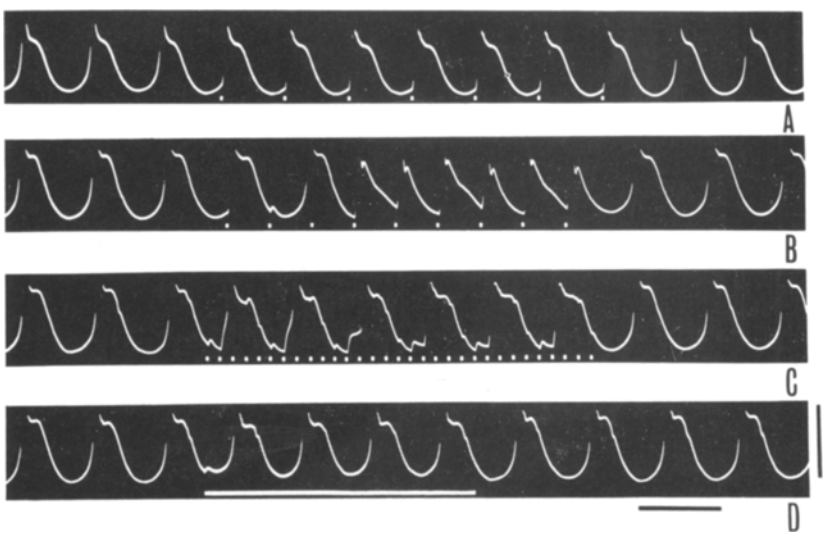

Intracellular recording from the ventricular region near the auriculoventricular valve of the heart of *Dolabella* showing the acceleratory effect of cardio-regulatory nerve stimulation at various frequencies. The white dashes (A, B and C) or line (D) under the trace indicate the stimulation. Calibration: 20 mV, 5 sec.

A) Interval between stimuli, 3.8 sec. Each stimulus is followed by an action potential. The individual EJP is not distinguishable from the spike.

B) Interval between stimuli, 2.5 sec. The second and third stimuli could not induce the action potential, but each stimuli following the third induced an action potential.

C) Interval between stimuli, 0.8 sec. The ratio of stimulation frequency to evoked action potential frequency is 1 to 5 or 6. The amplitude of the EJP varies according to various phases of the action potential cycle.

D) Interval between stimuli, 0.1 sec. The overall membrane potential is decreased by summation of EJP. The individual EJP is scarcely distinguishable, but on the falling phase EJP's appear as weak ripples.

seems to be due to the time relationship between EJP frequency and spontaneous cardiac rhythm, with regard to the membrane potential value, at the times when the evoked EJP's superimposed on the cardiac action potentials, and to refractoriness. Stimuli later than the second and third began to drive the cardiac action potential. There is a spurious impression of summation which is due to the slow rate of repolarization after each cardiac action potential. (The EJP evoked in the quiescent heart[6] showed that facilitation and summation hardly occurred at a frequency of less than 0.5 cps.) The induced action potential has a plateau of shorter duration and of lesser potential level than the normal one. Furthermore it is noticed that the size of action potential changes alternately, larger and smaller. With a slightly higher frequency (not illustrated) the smaller action potential failed to occur, as the stimulus fell on a slightly earlier phase of the repolarization. Simultaneously, the 'one action potential to one stimulus' relation disappeared and 'one to one' and 'one to two' relationships occurred alternately.

When the interval between stimuli became 0.8 sec, each induced action potential corresponded to 5 or 6 stimuli. An example is shown in Figure C. Large amplitude deflections which occurred just before the spike initiation, are considered to be due to a local response superimposed on the EJP. The amplitude of EJP is variable during one cycle of the action potential. In quiescent preparations[6] the amplitude of EJP's induced by such a stimulation series gradually increased and reached a steady level. Therefore, the variety of amplitude of EJP's may be attributed to the change of excitability and ionic flux in the muscle cell during one cycle of activity. The duration of the repolarizing phase of action potentials elongates during stimulation. It may be thought that the EJP's delay the repolarization of the membrane potential. In this case, intervals between action potentials before stimulation and during stimulation are about the same as in Figure A, measuring respectively 4.4 and 3.9 sec. Thus the effect of EJP's of such a high frequency on the rhythm of the action potential is weaker than at lower frequency (cf. Figure B), but its effect on the duration of the action potential is stronger.

When the stimulation frequency reached such a level that facilitation and summation occurred sufficiently to set up steady partial depolarization, the rhythm of action potentials was accelerated much as by overall depolarization of muscle membrane potential (unpublished work). This is shown in Figure D, in which the interval between the stimuli is 0.1 sec. At this frequency apparently the EJP shows summation, and the maximum potential of the cardiac cycle during stimulation becomes less than that of the normal action potential. The individual EJP's could scarcely be distinguished from each other in Figure D. The interval between normal action potentials and those intervals during stimulation are about 4.4 sec and 4.0 sec respectively. Effect of the EJP on the repolarization of action potential is not clearly evident, but the tendency is for each action potential during stimulation to have a prepotential like that of the pacemaker potential. It may be thought that the acceleratory effect at high frequency was brought about chiefly by depolarization of overall membrane potential, due to a summation of EJP's. It was observed in many cases that, in the range of stimulation frequencies at which the EJP is long enough to overlap, increase in stimulation frequency caused increased depolarization and increase in the action potential frequency. Probably the depolarization caused by summation of EJP's, as in the case of depolarizing current applied to the muscle cell, diminishes the potential difference between the maximum hyperpolarizing potential and the threshold potential.

Thus, the acceleratory effect of EJP's on the rhythm of action potentials seems to be due to different modes of action: 1. Individual low frequency EJP's evoke a corresponding action potential, and 2. summation of high frequency EJP's, by depolarizing the overall membrane potential, increases the frequency of the spontaneous action potentials. The former is illustrated clearly in Figures A and B, and the latter in Figure D. Interaction between the two modes produces a complex effect at intermediate frequencies (Figure C)[9].

Zusammenfassung. Nachweis, dass die Reizwirkung des Herz-Akzeleratornerven beim Molusken *Dolabella auricula* von der Reizfrequenz abhängt. Elektrische Ableitungen von einzelnen Herzkammerzellen zeigen, dass bei niedriger Reizfrequenz jedes nicht-summative «excitatory junctional potential» ein Aktionspotential des Herzens hervorruft.

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Photoperiod Manipulation to Control Diapause in the Pink Bollworm, *Pectinophora gossypiella*

Light breaks introduced during the period of darkness in a 24-h photoperiodic regimen have a marked effect on continued growth and development or on the induction of diapause in some species of insects[1–12]. The present paper reports further information about light breaks that may prove useful to those who are attempting to develop this phenomenon for the control of lepidopterous pests. The pink bollworm, *Pectinophora gossypiella* (Saunders), an important pest of cotton, was chosen as the test insect because the diapause of this species is so highly sensitive to manipulated photoperiods[9,13–17].

Eggs from adults reared in LL (continuous light) were shipped by air from Brownsville, Texas, to the laboratory at Beltsville, Maryland. There the freshly-hatched larvae were placed in 1-dram glass vials (15 × 45 mm) three-quarters filled with artificial medium[18], and the vials were then stoppered with cotton plugs. Supplementary medium was added on the tenth day. The vials were divided randomly into 8 lard can containers[19] equipped with 5-watt fluorescent lights that gave 150 lux at insect level and placed in a dark room maintained at 26 ± 1.5 °C. The lights in the containers were programmed for the regimens listed in the Table. Insects that had not pupated in 51–55 days were considered to be in diapause. The results are given in the Table and may be summarized as follows:

1. When a short day (LD 12:12) was used, diapause was found to be reduced by the introduction of 15 l-min