Multiple Intracellular Recording from Atrial and Sino-atrial Cells: Correlation with Contractile Tension.* (22655)

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(Introduced by T. A. Loomis.)
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In the course of microelectrode studies of rabbit atria in this laboratory it was noted that cells in the region of the sino-atrial node failed to respond to as high frequencies of electrical stimulation as did atrial cells. The present study was undertaken to investigate this phenomenon by means of simultaneous recording of atrial and sino-atrial cell action potentials. An effort was made to correlate the contractile response with the electrical activity of atrial muscle.

Methods. Virgin female albino rabbits were sacrificed by a blow on the neck and quickly exsanguinated. The thorax was opened and the entire heart quickly removed and dropped into oxygenated Tyrode solution, as modified by Hoffman and Suckling(1). In this solution, through which oxygen (95%)-carbon dioxide (5%) constantly bubbled, the combined atria were carefully separated from the ventricles and then mounted in a Plexiglas bath containing Tyrode solution. The bath was oxygenated through a diffusion dish which was incorporated into the chamber. The nutrient solution constantly flowed through at a rate of 3 cc per minute. The right atrial appendage was fastened to 2 silver hooks which also served as the stimulating electrode. The connective tissue in the septal area was secured to the arm of a sensitive strain gauge (Grass force displacement transducer), and the left atrium was allowed to float freely in the chamber. Stimulation was applied by means of a Grass Model 3C Stimulator. The output was passed through an isolation transformer to reduce the shock artifact. The signal produced by distortion of the strain gauge arm during contraction, was fed into a preamplifier, which in turn supplied the input to a DC amplifier of a Sanborn 4 channel recorder. Transmembrane potentials were determined with two cathode follower circuit units(2), using simultaneously 2 glass capillary electrodes with tip diameters of 0.5 μ or less. One electrode was used in the atrial myocardium exclusive of pacemaker region, while the other was used to explore the pacemaker area. Each of these circuits was connected to a separate DC amplifier in a Sanborn 4 channel recorder. The temperature of the bath was maintained between 28-30°C in order to reduce the spontaneous rate, lengthen the duration of the action potential and to maintain better contractility.

Results. Tracings of typical records obtained during simultaneous impalement of atrial and sino-atrial cells are seen in Fig. 1. The dotted line represents the zero potential level. Sino-atrial cell firing can be recognized by the slow diastolic prepotential development as noted in the upper portion of the tracings. After a control period, electrical stimulation was initiated and maintained for 1 to 3 seconds. Recovery represents the beginning of spontaneous activity following electrical stimulation. At the 2 frequencies depicted (3.75 and 4.6 stimuli per second), the atrial cell follows, while the sino-atrial cell follows at the lower but not at the higher frequency. In general, the amplitude of atrial cell action potentials was 90-120 mv, while that of sino-atrial cells was lower (50 to 70 mv). The lower values for sino-atrial cell action potentials are in agreement with the reports of other investigators working with rabbit atria(2) and frog sinus venosus(3,4).

Fig. 2 summarizes the results obtained on frequency of response of atrial and sino-atrial cells versus frequency of electrical stimulation applied. Blackened circles (sino-atrial responses) and open circles (atrial cell responses) represent the arithmetic mean of 2

* Supported in part by grant from National Heart Institute, U.S.P.H.S. Presented before the Soc. for Exp. Biol. and Med. at the AAAS 37th meeting June 1956 at University of Washington.

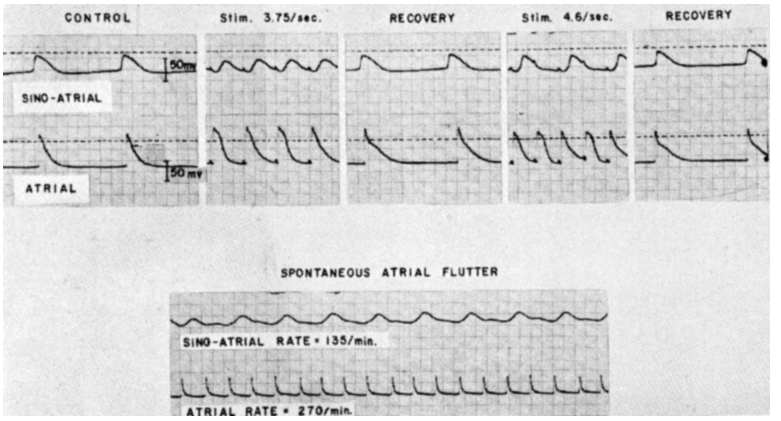

FIG. 1. Simultaneous recording of sino-atrial and atrial cell action potentials during electrical stimulation and spontaneous atrial flutter. Dotted line represents zero potential level. Each small division represents one mm. Paper speed, 50 mm/sec.

to 18 determinations. At lower frequencies the minimum number of determinations was 4 to 5, with a maximum at 18, while at higher frequencies (8-16 stimuli per second) a minimum of 2 determinations were made. The vertical bars are ranges of responses at any one frequency. Inspection of the graph discloses a linear relationship (1:1) for atrial cells at stimulation frequencies as high as 10 per second. The slope of the line drawn through the open circles is unity. Sino-atrial cells fail to follow rates as low as 4 per second. Stimulation frequencies beyond this point resulted in an integral ratio of atrial to sino-atrial cell responses; being 2:1 up to a frequency of 7.5 per second, then 3:1 and 4:1 at higher frequencies.

A recording taken at a time of spontaneous atrial flutter is shown in Fig. 1. There is a 2:1 ratio between atrial and sino-atrial cell firing, a phenomenon which parallels the results obtained from electrical stimulation.

Shown in Fig. 3 are simultaneous recordings of action potentials of an atrial cell and the tension developed by the right atrium. At the onset of electrical stimulation there is a decrease in tension (3rd beat, Fig. 3). This has been reported for isolated papillary muscle by Gold and Cattell(5). As stimulation was continued, the tension alternated in a regular manner such that a beat in which high tension developed was followed by a beat of low tension. Both the high and low tension contractions exhibited a positive staircase effect(6,7) during the applied electrical stimulus. When stimulation was stopped, there was a marked increase in tension on recovery with the occurrence of a negative staircase effect. It is of interest to note the repolarization phase of atrial cell action potentials during electrical stimulation (4th to 6th and 9th to the 13th beats in Fig. 1, 3rd to the 12th beats in Fig. 3). Alternation in repolarization configuration can be seen; every other beat exhibited an exponential repolarization in contrast to the more sigmoid repolarization of the intervening beats. In Fig. 3, alternation becomes apparent during continued electrical stimulation. The tension is greater in those cycles where repolarization

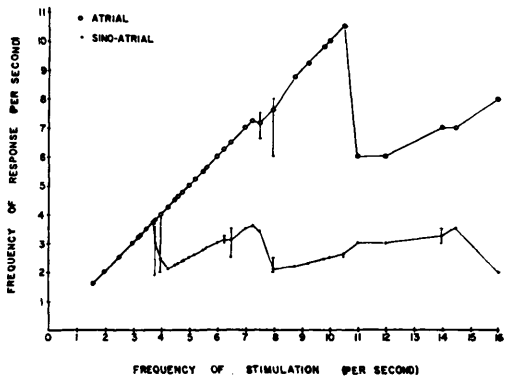

FIG. 2. Graph summarizing data of simultaneous recording of atrial and sino-atrial cell action potentials during electrical stimulation. Open circles, atrial cell responses; blackened circles, sino-atrial cell responses. Ordinate: frequency of electrical stimulation. Abscissa: frequency of response.

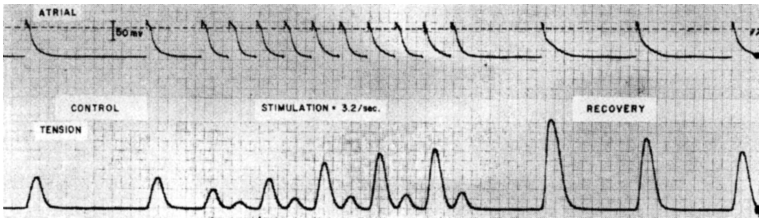

FIG. 3. Simultaneous recording of atrial cell action potentials (upper tracing) and tension developed by right atrium (lower tracing). Dotted line represents zero potential level. Each small division represents one mm. Paper speed, 50 mm/sec.

was exponential rather than sigmoid (5th, 7th, 9th and 11th beats in Fig. 3). Post-stimulation potentiation of tension is accompanied by a repolarization curve which is considerably longer in duration than those of the control action potentials or those occurring during electrical stimulation (7th and 15th beats in Fig. 1, 13th beat in Fig. 3). Tension development and action potential configuration returned to control values after 4 or 5 spontaneous beats.

Discussion. The difference in the follow rates of sino-atrial and atrial cells is to be expected on the basis of the much longer duration of the action potentials of sino-atrial cells(2,8,9). An electrical stimulus falling during the earlier phases of repolarization fails to excite sino-atrial cells, while on the same time scale the stimulus would excite atrial cells which have already returned to the polarized state. The sino-atrial region appears to act as pacemaker normally because it is usually in a partially depolarized state (2,8,9) and has the greatest tendency to undergo cyclic spontaneous depolarization. Certainly, pacemaker activity cannot be explained on the basis of refractory period, since the experiments reported here indicate a longer refractory period for sino-atrial cells. The same discrepancy between atrial and sino-atrial cells has been observed when a relatively rapid "flutter" has been induced in the preparation. When the rate of stimulation exceeded that rate at which the sino-atrial cells could follow, it was observed that alternation in both electrical and mechanical phenomena occurred. That is, the repolarization of atrial cells alternated between a sigmoid and exponential curve. At the same time, total contractile tension alternated in magnitude. It was observed that high tension was associated with the exponential type repolarization curves whereas low tension matched the sigmoid type repolarization. Inspection of simultaneous tracings of atrial and sino-atrial spikes showed that effective depolarization in sino-atrial cells also was coincidental with the exponential repolarization curve in atrial cells. In those experiments in which simultaneous atrial and sino-atrial electrical events and contractile tension all were observed, the same correlation was observed within limits. The cause-effect relationship is not yet clear. However, at higher frequencies a 4:1 ratio between atrial and sino-atrial response may be evident without a similar pattern in tension magnitude. It is felt that the correlation between sino-atrial cell firing and tension, when tension is alternating at lower frequencies, probably is coincidental. On the other hand, the relationship between atrial cell repolarization and tension, which is a consistent finding in this preparation, appears to be valid at all frequencies, although they are not related in any simple way. Further investigation of this phenomenon is in progress.

Post-stimulation potentiation and post-extrasystolic potentiation in the heart have been studied by other investigators. The studies of Rosin and Farah(10) were conducted on isolated rabbit atria and ventricles; those of Furchgott and Sleator(11) on guinea pig atria. Changes in contractility were qualitatively similar to those reported here. Neither of these authors attempted to relate contractility to transmembrane potentials. This has been done recently by Hoffman, Bindler and Suckling(12) using cat papillary muscle. However, the post-extra-

systolic potentiation observed by the latter investigators was not accompanied by a significant change in membrane repolarization.

Summary. 1. Cells in the region of the sino-atrial node of rabbit are unable to respond to as high a frequency of stimulation as those cells not included in the pacemaker area. 2. The same phenomenon has been observed during rapid atrial arrhythmias in the absence of electrical stimulation. 3. The magnitude of the contractile tension developed by the atrium is related to repolarization of atrial cell action potentials, being greater for those demonstrating exponential rather than sigmoid curves. This is a consistent finding occurring during periods of electrical stimulation and with the first few spontaneous beats following stimulation, but the tension and action potential repolarization are not related to each other in any simple way.

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Received June 29, 1956. P.S.E.B.M., 1956, v93.

Effect of DPPD, Methylene Blue, BHT, and Hydroquinone on Reproductive Process in the Rat.* (22656)

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Although several compounds have been found to prevent or cure certain vit. E deficiency symptoms, such as brown discoloration of the uterus and cloudy swelling of the convoluted tubules of the kidney, only 2, diphenyl-p-phenylenediamine (DPPD) and methylene blue, have been reported to substitute for tocopherol in preventing resorption-gestation in vit. E-deficient female rats. In our laboratory also, preliminary experiments indicated that methylene blue and DPPD gave slight positive responses under the conditions of our standard vit. E bioassay procedure(1). Consequently, we were led to investigate this apparent vit. E activity further.

Methylene blue. The results of vit. E bioassays in which the responses of 5 levels of methylene blue were compared with five levels of standard, *d*-α-tocopherol. showed median fertility doses (MFD) of 36 mg and 0.29 mg respectively. Thus, methylene blue has less than 1% the activity of α-tocopherol. However, during the course of these experiments, Moore, Sharman, and Ward(2) reported that under the conditions of vit. E bioassay in their laboratory, methylene blue was completely negative. The bioassay procedures used at Cambridge and by us were practically identical except for length of time of depletion. Moore used 5 months and we used about 5 weeks. Consequently, the slight positive response we obtained for methylene blue may have been due to its protective or sparing effect on the small, residual quantities of α-tocopherol still remaining in the tissues of our animals after only 5 weeks deple-

* Communication No. 233.