Ultraviolet-Induced Alterations of Beat Rate and Electrical Properties of Embryonic Chick Heart Cell Aggregates

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ABSTRACT Embryonic heart cell aggregates were irradiated with ultraviolet light at wavelengths between 260 and 310 nm. Spontaneous beat rate was monitored with the aid of a closed-circuit TV camera and, in separate experiments, electrophysiological changes were assayed by intracellular recording. The characteristic response of 7-day aggregates was an increase in spontaneous beat rate to a maximum plateau level, followed by a rather abrupt cessation of beating. Intracellular recordings during irradiation showed a marked decline in the maximum rate of rise, overshoot, and repolarization phase of the action potential, and a significant change in threshold toward zero. The action spectrum for the termination of beating peaked between 290 and 295 nm; it fell off sharply at longer wavelengths and more slowly at shorter wavelengths. The maximum increase in beat rate was increasingly greater for shorter wavelengths and exhibited no peak in the wavelength range investigated. The sensitivity of aggregates to 295-nm light, as measured by the inverse of irradiation time required to terminate beating, decreased with increasing aggregate size and external potassium concentration, was relatively independent of temperature, and increased with embryonic age. The ultraviolet-induced increase in beat rate and termination of beating are attributed to separate complementary processes, a depolarization of the membrane, and a decline in "fast" sodium conductance.

INTRODUCTION

While a number of recent investigations have dealt with a description of molecular mechanisms responsible for excitability in nerve (Hille, 1972; Bezanilla and Armstrong, 1974), few attempts have been made to define these processes in cardiac muscle. A variety of optical techniques have been useful in such studies on nerve (see review by Cohen, 1973). One of these techniques, irradiation with ultraviolet (UV) light, was employed in conjunction with the powerful voltage clamp technique in experiments with frog nodes of Ranvier (Fox and Stämpfli, 1971; Fox, 1972, 1974 *a, b*) and with lobster giant axons (Oxford, 1974; Oxford and Pooler, 1975). It was shown that specific ionic conductances, particularly sodium, decreased during UV irradiation, leading ultimately to conduction

block. These effects were found to be most dramatic at wavelengths characteristic of absorption by proteins.

In contrast to neural preparations, cells of the early embryonic heart appear to be devoid of a "fast" tetrodotoxin (TTX)-sensitive sodium conductance mechanism (McDonald et al., 1972). The rhythmic spontaneous action currents of the 4-day heart, for example, are carried primarily via a "slow" sodium conductance (Sperelakis, 1972). Later in development, membrane selectivity changes to involve a fast sodium-specific mechanism (DeHaan et al., 1975). Recent work (Sachs and DeHaan, 1973; DeHaan and Fozzard, 1975) has led to the preparation of a culture system of spheroidal aggregates of electrically coupled embryonic heart cells in which both passive and excitable properties can be investigated in detail. Furthermore, the geometry, size, and developmental age of these preparations can readily be manipulated (DeHaan et al., 1975).

In the present work, UV radiation was applied to heart cell aggregates as an initial step in an investigation of the molecular mechanisms and development of the excitable state in the cardiac myocyte membrane. The specific questions we wished to answer were: (*a*) Does UV irradiation of aggregates inhibit excitability by altering ionic permeability mechanisms similar to those in nerve tissue. (*b*) Is the inhibition of beating maximal at wavelengths similar to those described for neural preparations. (*c*) Is the UV sensitivity of aggregates a function of their developmental age, as is the case for their sensitivity to TTX (McDonald et al., 1972). The experiments reported here address these questions.

METHODS

Heart Cell Aggregates

Hearts from White Leghorn chick embryos incubated 4, 7, or 14 days were excised and trimmed of extraneous tissue. The apical portion of the ventricles in the case of 7-day hearts, or whole hearts from 4- and 14-day embryos were dissociated into their component cells with trypsin (DeHaan, 1967, 1970). Aggregates of dissociated cells were prepared as described previously (McDonald et al., 1972; Sachs and DeHaan, 1973). Briefly, suspensions of 5×10^5 cells in 3 ml of medium (21212) were added to 25-ml Erlenmeyer flasks and gassed with a 5% CO_2, 10% O_2, 85% N_2 mixture. Flasks were sealed with silicone rubber stoppers and placed on a gyratory shaker at 37°C. Aggregation took place during 24–72-h gyration at 50 rpm ($1\frac{1}{4}$-inch stroke). Aggregates were transferred from flasks to 35-mm tissue culture dishes (Falcon Plastics, Oxnard, Calif.) where they were positioned at distances 0.5 mm or more apart and allowed 2 h (under an atmosphere of 5% CO_2, 10% O_2, 85% N_2) to attach to the dish bottom.

Solutions

Aggregates were prepared in medium 21212, an enriched form of medium 818A (DeHaan, 1970). This medium contains 25% M 199 (Grand Island Biological Co., Grand Island, N. Y.), 2% heat-inactivated selected horse serum (Colorado Serum Co., Denver, Colo.), 4% fetal calf serum (Grand Island), 68.5% potassium-free Ham's F12 (Grand Island), and 0.5% Gentamycin (Schering Corp., Bloomfield, N. J.). Because of the strong UV absorption of medium 21212, aggregates were washed and maintained in modified Earle's balanced salt solution (BSS) during irradiation and subsequent observation. The BSS contained the following components (mM): NaCl, 116.0; $MgSO_4$, 0.8; NaH_2PO_4, 0.9;

$NaHCO_3$, 26.2; $CaCl_2$, 1.8; and glucose, 5.5. The potassium concentration of the salt solution was adjusted to 1.0 mM for most experiments by the addition of salt solution containing 100 mM KCl.

Maintenance and Recording of Beating

A tissue culture dish with aggregates adhering to the bottom was transferred to a heated platform on the stage of a dissection microscope. A toroidal gassing ring surrounding the dish maintained a constant atmosphere of 5% CO_2, 10% O_2, and 85% N_2 and a pH of 7.3. The dish was rinsed once and medium 21212 was replaced by 2 ml of potassium-free BSS. Potassium concentration was adjusted to 1.0 mM by adding 40 μl of BSS containing 100 mM KCl, and the dish was brought to a final volume of 4 ml (4.00 g) with BSS, as measured by weighing the dish on a balance. To compensate for evaporation, the salt concentration was maintained by continuous slow addition of distilled water (Sage Pump, model 255-3, Sage Instruments Div., Orion Research Inc., Cambridge, Mass.). The volume was checked at half-hour or hourly intervals by weighing the dish, and the pumping rate was adjusted accordingly. Under these conditions the greatest change in salt concentration for any experiment was 6%/h. The temperature of the bathing solution was continuously monitored by a telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio, model 43) with a thermistor probe and was controlled by adjusting the current supplied to the heating coil from a DC power supply (Hewlett-Packard Co., Palo Alto, Calif., model 6205B). Most experiments were carried out at a temperature of 30°C, at which level the variation across the dish bottom was less than 1°C.

Rhythmically beating aggregates were viewed at a magnification of × 40 and their sizes were measured with an ocular reticle whose smallest division represented 5.1 μm. Measurements could be made accurately to about two divisions. Total cell surface area of aggregates was estimated by assuming that all cells were spherical in shape and had volumes of 817 μm^3 and surface areas of 423 μm^2 (DeHaan, unpublished). The number of cells per aggregate was estimated assuming that 80% of the aggregate volume was occupied by cells and 20% by extracellular space, based on the data of McDonald and DeHaan (1973). Aggregate volumes were calculated from measurements of the major and minor axes using the equation for the volume of a prolate spheroid (DeHaan and Sachs, 1972). Total cell surface area was calculated as the product of the number of cells per aggregate and the surface area per cell. Aggregates of 180–240-μm diameter ($1.3 - 3.0 \times 10$-μm^2 total cell surface area) were selected, with the exception of those control experiments concerned with determining the dependence of UV sensitivity on size.

A miniature closed-circuit TV camera (Panasonic, model WV-400P, Matsushita Communication Industrial Co., LTD., Yokohama, Japan) was positioned on one of the oculars of the dissecting microscope, freeing the other ocular for normal viewing and size determinations. At a magnification of × 40, beating of heart cell aggregates as small as 100 μm in diameter could easily be seen on a TV screen (Panasonic, model TR-413V). Individual contractions were recorded by placing a phototransitor sensor (Fairchild Industrial Products Div., Fairchild Industries, Winston-Salem, N. C., FPT 100) over a portion of the aggregate image which changed its reflectance with each beat. Amplified pulses (Wolf and DeHaan, 1971) were displayed on an oscilloscope (Tektronix, Inc., Beaverton, Ore., model R5103N/D13) and recorded on an instrumentation recorder (Hewlett-Packard, model 3960) at a tape speed of $^{15}/_{16}$ in/s and on a strip chart recorder (Bausch and Lomb, Inc., Rochester, N. Y., model VOM5) at 20 in/min.

Light Source and Optics

White light from a 1,000-W high pressure xenon arc lamp (Hanovia Lamp Div., Englehard Hanovia, Inc., Newark, N. J., model 976-C1), mounted in a forced-air ventilated

aluminum housing (Electro Powerpacs, model 371, Electro Powerpacs Corp., Cambridge, Mass.), was focused by a quartz condenser onto the entrance of a 1/4-m grating monochromator (Jarrell Ash Div., Fisher Scientific Co., Waltham, Mass., model 82-410). Fig. 1 illustrates the apparatus. The 2-mm slits used in these experiments provided a half-maximum intensity bandwidth of 3.3 nm. Selected wavelengths were reproducible to within 0.2 nm using the digital wavelength readout and were calibrated with a mercury lamp.

The output of the monochromator was collimated by a quartz lens, reflected at 45° by a MgF_2-coated aluminum front surface mirror (Microcoatings, Inc., Waltham, Mass.) and focused onto the preparation by a third quartz lens. By masking the monochromator exit slit, the focused beam was restricted to an area of about 350 × 350 μm^2 at the plane of the tissue culture dish bottom.

A metal shutter was used to block the light when not in use. A thermopile light detector (Sensors, Inc., Ann Arbor, Mich., model C1), whose output was amplified and recorded on a second channel of the tape recorder, was positioned near the shutter to record the time-course of irradiation.

Each aggregate was positioned in the UV light path by temporarily switching the monochromator to visible light (640 nm) and manually centering the aggregate at the brightest spot. For measurements of action spectra the sequence of stimulus wavelengths was selected using a table of random numbers, and for all other experiments the wavelength of UV light was 295 nm.

Electrophysiology

Action potentials were recorded intracellularly from 7-day aggregates during irradiation at 295 nm or during the application of depolarizing current. All impalements were made at 30°C in BSS containing 1.0 mM potassium. Glass micropipettes were filled with 3 M KCl by the method of Tasaki et al. (1968) and had DC resistances of 20-40 MΩ. Intracellular electrodes were coupled to capacitance-compensated electrometer amplifiers (Instrumentation Laboratory, Inc., Lexington, Mass., Picometric, model 181) and the extracellular

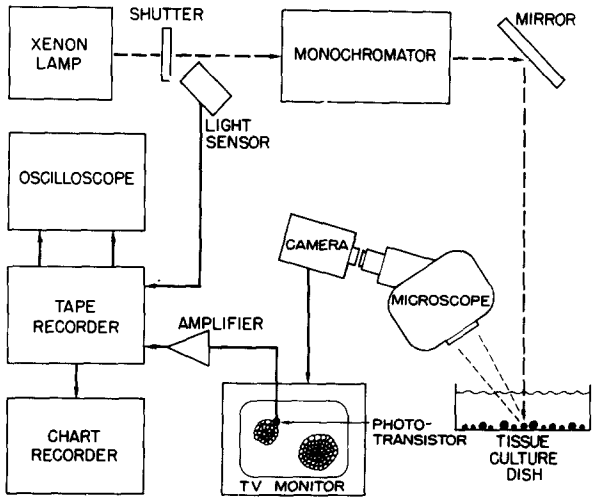

FIGURE 1. Schematic diagram of the apparatus for irradiation and recording. The heavy dashed lines represent the light path, and the heavy solid lines represent electrical wiring. Lenses have been omitted for clarity.

medium was connected to ground by an agar bridge. For current injection an aggregate was impaled with two microelectrodes, one as a current source and one for potential recording. Current pulses of 10–20-s duration were held constant (to within about 15%) by a 100-MΩ resistor in series with the voltage source.

Impalements were monitored on the storage oscilloscope and data were recorded at $3^3/_4$ in/s on the FM recorder. At this tape speed the frequency response was flat to 1.25 KHz. Parameters measured from action potentials photographed directly from the oscilloscope during an experiment or later from playback of the tape did not differ, even at sweep speeds of 1 ms/division. Data recorded on tape were photographed with an oscilloscope camera (Nihon, model PC-2A, Nihon Kohden Kogyo Co., LTD., Tokyo, Japan), and action potential parameters were calculated from enlarged images digitized on a Hewlett-Packard, model 9864A digitizer.

Data Analysis

Two parameters were chosen to assay the magnitude of UV effects on spontaneous beating of nonimpaled aggregates. The irradiation time required to stop beating (t_s) was determined by a stopwatch while viewing the contractions or from the strip chart records. The maximum percent change in beat rate, $(R_{max} - R_o)/R_o$, was used to express the magnitude of the light-induced increase in beat frequency.

Action spectra were corrected for spectral variations in the intensity of light reaching the preparation. Table I lists the components which were combined to yield a correction factor at each wavelength. The balanced salt solution transmission spectrum was determined from media taken from six dishes in which aggregates had been irradiated. Values for percent transmission were measured by a UV-visible spectrophotometer (Varian Associates, Palo Alto, Calif., model 635) with 1-mm slits and were corrected for the difference in cuvette (1.0 cm) and medium (0.42 cm) optical path lengths. Relative values for monochromator and optics transmission and for lamp output intensity were calculated from published curves and were identical to those used by Oxford and Pooler (1975). The major part of the correction was due to a decrease in lamp intensity at shorter wavelengths.

TABLE I

CALCULATION OF LUMPED INTENSITY CORRECTION FACTORS

Wavelength (nm)	260	265	270	275	280	285	290	295	300	305	310
Salt solution transmission* (%)	86.0 ±2.6	86.0 ±2.6	86.0 ±2.5	86.1 ±2.5	86.4 ±2.5	86.9 ±2.5	87.5 ±2.5	88.1 ±2.4	88.6 ±2.4	89.0 ±2.2	89.4 ±2.2
Relative salt solution transmission (%)	96.2	96.2	96.2	96.3	96.6	97.2	97.9	98.5	99.1	99.6	100
Relative transmission of monochromator and optics (%)	86.0	88.6	90.0	93.7	96.7	98.0	99.6	100	100	99.0	97.7
Lamp relative output intensity (%)	50	55	58	65	70	76	80	85	90	95	100
Lumped intensity correction factor‡	2.36	2.08	1.93	1.66	1.49	1.35	1.25	1.17	1.10	1.04	1.00

* Values corrected for the difference in cuvette (1.0 cm) and medium (0.42 cm) optical path lengths (mean ± SE, $n = 6$).

‡ Calculated as the inverse of the product of the salt solution relative transmission, monochromator and optics relative transmission, and lamp relative output intensity; normalized to 1.0 at 310 nm.

For impaled aggregates the maximum rate of rise ($+\dot{V}_{max}$) was determined at an oscilloscope sweep speed of 0.2 ms/division. The instantaneous beat rate was calculated as the inverse of the time between successive maximum diastolic potentials (MDP's), and the threshold for firing was taken to be the intersection of lines tangent to the slope of the diastolic depolarization and to the point of maximal upstroke velocity of the action potential.

RESULTS

The characteristic effect of irradiating heart cell aggregates at wavelengths between 265 and 305 nm was an increase in beat rate to a maximum plateau level followed by an abrupt cessation of beating. At 260 and 310 nm where the effect was minimal, beating continued until the experiments were terminated after 10–15 min. Fig. 2 illustrates the time-course of light-induced effects for several wavelengths between 295 and 310 nm and the variability of a nonirradiated control. The coefficient of variation for this "worst case" control example was 3.3% for a total of 81 measurements (every 15 s) over a 20-min period.

Action Spectra

Action spectra for the light-induced termination of beating and increase in beat rate of 7-day aggregates are illustrated in Fig. 3. Means (±SE) of the inverse of the irradiation time to stop beating ($1/t_s$) and the maximum percent change in beat rate $(R_{max} - R_o)/R_o$ are plotted versus wavelength for a total of 97 aggregates from 11 dishes. The action spectrum for the termination of beating peaked between 290 and 295 nm and fell off sharply at longer wavelengths and more slowly at shorter wavelengths. In contrast, the maximum increase in beat rate was increasingly greater for shorter wavelengths and exhibited no peak in the wavelength range investigated. The lack of agreement between these two action spectra suggests that different species of chromophores may be responsible.

Aggregate Size

Size was an important factor in determining the response of heart cell aggregates to UV irradiation. As illustrated in Fig. 4 larger aggregates were less sensitive than smaller ones, suggesting a possible shielding of inner cells by more superficial cells. Also, small aggregates tend to flatten more rapidly on the dish than larger spheres. Thus at the time of irradiation they might be expected to have had proportionally more surface area exposed. It may be for this reason that the two smallest aggregates (both less than 150-μm diameter) deviate sharply from an otherwise smooth exponential relationship. In view of the significant dependence of UV sensitivity on aggregate size, aggregates of a narrow range of diameters were selected for all other experiments.

External Conditions

External potassium concentration was also found to influence the time to stop beating. Using 0.5 mM steps of potassium concentration, the UV sensitivity of 38 aggregates was inversely related to potassium concentration. Aggregates in 2.5 mM potassium required 2.14 ± 0.07 min (mean ± SE, n = 7) to stop beating

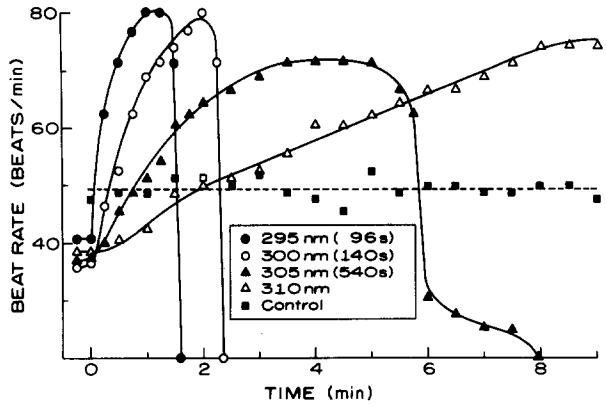

FIGURE 2. Time-course of the light-induced effect on 7-day aggregates. Each curve illustrates the result of irradiating a single aggregate at 295, 300, 305, or 310 nm. The times to stop beating are presented at each wavelength. The aggregate irradiated at 310 nm was still beating after 10 min when the experiment was terminated. Potassium concentration was 1.0 mM, mean temperature was 30.4 ± 0.5°C, and mean aggregate diameter was 203 ± 3 μm.

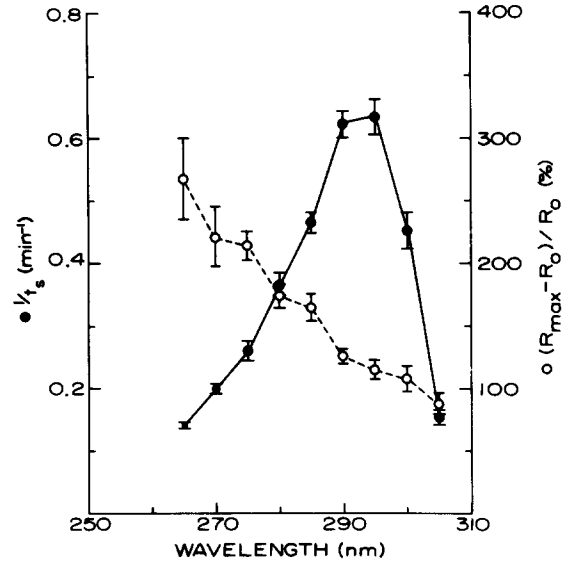

FIGURE 3. Action spectra for the light-induced effects on 7-day aggregates expressed as the inverse of the time to stop beating ($1/t_s$) and as the maximum percent change in beat rate $(R_{max} - R_0)/R_0$ versus wavelength. Values are means (± SE). For increasing wavelength n was 5, 10, 9, 10, 9, 11, 24, 9, and 10. All values have been corrected for spectral variations in the intensity of light reaching the preparation. For all experiments, potassium concentration was 1.0 mM, mean temperature was 30.9 ± 1.4°C and mean aggregate diameter was 205 ± 19 μm.

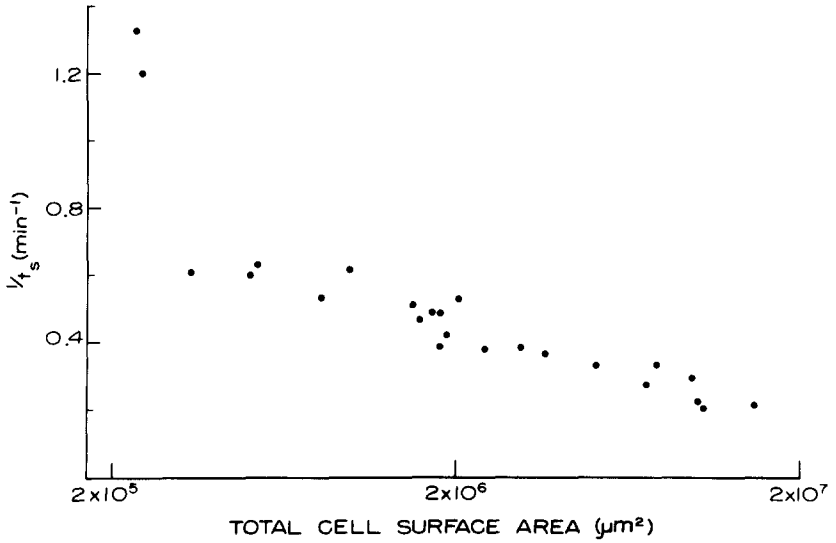

FIGURE 4. Sensitivity of 7-day aggregates to UV irradiation expressed as the inverse of the time to stop beating ($1/t_s$) versus total cell surface area. Each value represents the effect on a single aggregate, and all aggregates are from the same dish. External potassium concentration was 1.0 mM, mean temperature was 29.7 ± 0.4°C, and stimulus wavelength was 295 nm. An aggregate with total cell surface area of $1 \times 10^6 \mu m^2$ has a diameter of 167 μm.

while those in 0.5 mM potassium required only 1.25 ± 0.03 min (mean ± SE, n = 5). In contrast, temperature had little effect on aggregate sensitivity between 25 and 38°C. The inverse of the time to stop beating was slightly greater at minimum and maximum temperatures. During irradiation, fluctuations in temperature were never more than 1°C and usually were only 0.1–0.2°C. Furthermore, the local temperature in the vicinity of each aggregate differed by less than 1°C across the dish. Control experiments confirmed the fact that the macroscopic temperature of the bathing medium was not altered by the UV light itself. With one thermistor directly under the stimulus and another a few millimeters away, no measurable difference in temperature (0.1°C resolution) was recorded after 6 min of irradiation at either 285 or 800 nm.

Embryo Age

We found the sensitivity of heart cell aggregates to UV radiation to be dependent upon the developmental age of the chick embryos from which they were derived. Fig. 5 illustrates this dependence for time and rate parameters used to assay the light-induced effect. The results of two sets of experiments were pooled to calculate these curves. In one set a single dish contained 4-, 7-, and 14-day aggregates positioned in different sectors of the dish while in the second set aggregates of different ages were contained in separate dishes. For the latter experiments, conditions were equivalent in all dishes since mean temperatures and aggregate diameters for each dish were within 1 SD of one another, and potassium concentration was constant at 1.0 mM.

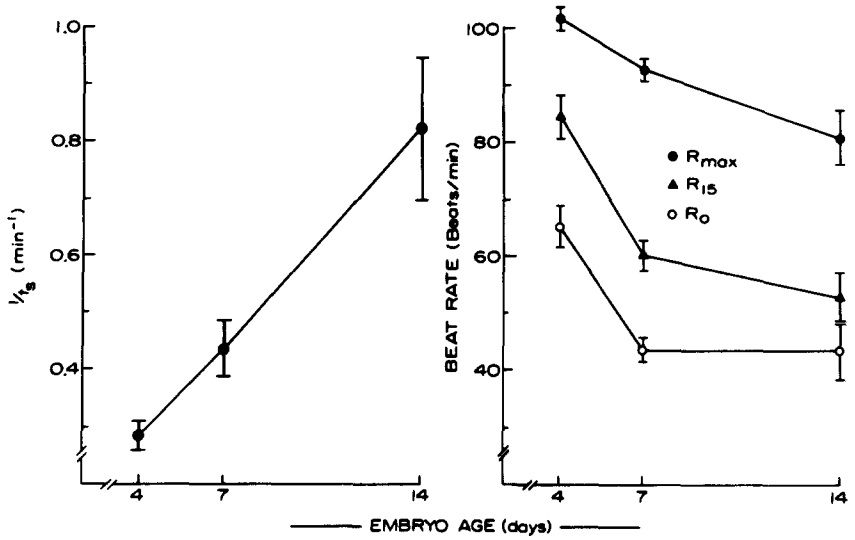

FIGURE 5. Sensitivity of aggregates to UV radiation as a function of embryo development. Time (left) and rate (right) parameters derived from the same aggregates are plotted versus embryo age. Values are mean (± SE). For increasing age n was 13, 16, and 11. External potassium concentration was 1.0 mM, mean temperature was 29.6 ± 0.2°C, mean aggregate diameter was 217 ± 3 μm, and stimulus wavelength was 295 nm.

The data shown in Fig. 5 indicate a 289% increase in sensitivity for $1/t_s$ between 4- and 14-days development but a 21% decline in sensitivity for the maximum beat rate during the same developmental period. When expressed as a percent change in beat rate the behavior is complicated because of the strong dependence of initial rate on developmental age. Interestingly, the fraction of the total increase in rate reached after 15 s of irradiation was significantly greater for the 4-day aggregates, being 55%, as opposed to 33 and 26% for the 7- and 14-day aggregates.

Reversibility

For 30-s periods of irradiation the spontaneous beat rate increased up to 60%. In six experiments this increase in rate consistently failed to decay during the subsequent 60 s after the light was turned off. However, slow partial recovery sometimes did occur over a period of hours after cessation of irradiation, if irradiation was terminated before an aggregate stopped beating. For example, one 7-day aggregate irradiated for 1 min increased its rate by 118%. Two minutes after irradiation had ceased its beating began to slow, and in 1 h it had recovered to within 35% of its original beat rate. Aggregates that were irradiated long enough to abolish beating did not recover, even after 24 h.

Electrophysiology

Ultraviolet irradiation at 295 nm had a dramatic effect upon action potentials recorded intracellularly from 7-day aggregates. Fig. 6 illustrates the behavior at

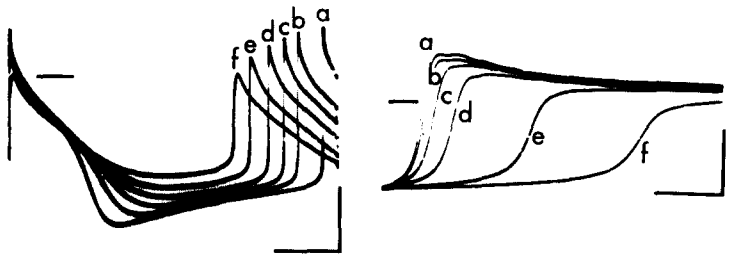

FIGURE 6. Action potentials recorded intracellularly from a 7-day aggregate during irradiation at 295 nm. External potassium concentration was 1.0 mM, mean temperature was 29.3°C and size was 184 × 204 μm. Scales represent 40 mV, 0.2 s (left), and 2 ms (right). Horizontal lines represent 0 mV. (Left) Action potentials *a–f* recorded at time 0 and 20, 40, 60, 80, 100, s of irradiation. (Right) Action potentials *a–f* recorded at time 0 and 20, 40, 60, 80, 90, s of irradiation.

progressive times during continuous irradiation. The major effects were the following: depolarization, a significant change of threshold toward zero, an increase in beat frequency, slowing of the repolarization phase, a reduction of the overshoot, and a marked decrease in the maximum rate of rise. No appreciable change occurred in the slope of the pacemaker potential. A quantitative comparison of these parameters is given in Fig. 7. With the exception of beat rate, each parameter has been normalized to its peak value before irradiation. In six other aggregates the behavior was similar to that illustrated in Figs. 6 and 7.

Although the instantaneous beat rate recorded intracellularly increased during irradiation in agreement with measurements made visually without microelectrode impalements, the maximum change was not as great during irradiation with intracellular recording. This discrepancy is most likely the result of a slight membrane leakage due to impalement which often caused the frequency of spontaneous beating to increase by more than 50% before irradiation was begun. For example, the beat rate for the aggregate represented in Fig. 7 was 41 beats/min before impalement (as measured visually). Immediately after impalement the rate increased to 89 beats/min and 3 min later it had declined to a stable rate of 61 beats/min, 6 s before irradiation. Using the initial beat rate before impalement, the maximum percent change was actually 107% which agrees well with the mean value at 295 nm in Fig. 3. To control for the possibility that the observed depolarization might result from a poor impalement, we recorded constant potentials in all aggregates for 1-3 min before and up to 2 min after irradiation.

The values illustrated in Fig. 7 were obtained after a 3-min control recording in which no action potential parameter, with the exception of beat rate, changed more than 5% from the mean. After 107 s of irradiation, spontaneous firing ceased at a MDP of −59 mV and the potential came to rest at −46 mV. At this time the light was turned off and this steady potential was recorded for the next 2 min. Some aggregates stopped beating at MDP's more negative than −59 mV, but all stabilized immediately at about the same potential. By applying brief current pulses up to 9 nA through the recording electrode, weak action poten-

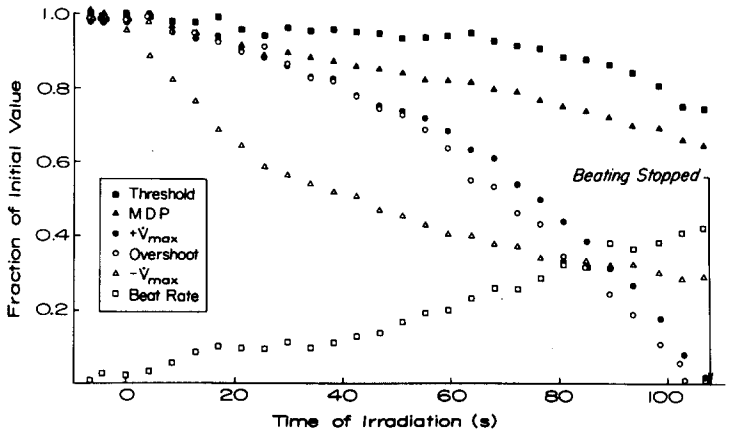

FIGURE 7. Parameters calculated from action potentials recorded intracellularly from a 7-day aggregate (same aggregate as in Fig. 6) during irradiation at 295 nm. Each point represents a measurement from an action potential selected at intervals from the tape. Instantaneous beat rate was measured as the inverse of the time between successive maximum diastolic potentials (MDP's) and is plotted as the fractional change from the initial rate, where 1.0 represents a 100% change. The maximum rate of rise is represented by $+\dot{V}_{max}$ and the maximum rate of repolarization after the plateau phase by $-\dot{V}_{max}$. Initial and final values were as follows: threshold, −67 and −50 mV; MDP, −91 and −59 mV; overshoot, +29 and −10 mV; $+\dot{V}_{max}$, 134 and 2 V/s; $-\dot{V}_{max}$, −479 and −137 mV/s; and instantaneous beat rate, 61 and 87 beats/min.

tials could be stimulated in these aggregates that had just stopped beating; however, the peak-to-peak amplitudes were usually less than 40 mV and the maximum rates of rise less than 1 V/s.

In order to distinguish between those changes in action potential characteristics due directly to UV and those produced secondarily by light-induced depolarization, sufficient current was injected into an aggregate through a second intracellular microelectrode to produce a prolonged period of stable depolarization, during which several spontaneous action potentials were recorded. Fig. 8 compares $+\dot{V}_{max}$ and overshoot for five irradiated and three current-depolarized aggregates. Both the overshoot and $+\dot{V}_{max}$ declined significantly more during ultraviolet irradiation than could be accounted for by depolarization alone. For example, when the MDP was reduced to −60 mV by UV, the overshoot fell 50% more, and $+\dot{V}_{max}$ 39% more than during current-induced depolarization to the same level. Furthermore, during irradiation aggregates did not beat spontaneously at MDP's more positive than −58 mV, while some aggregates depolarized by current injection continued to beat at potentials as low as −48 mV.

DISCUSSION

The characteristic response of 7-day heart cell aggregates to ultraviolet irradiation was an increase in spontaneous beat rate to a maximum plateau level,

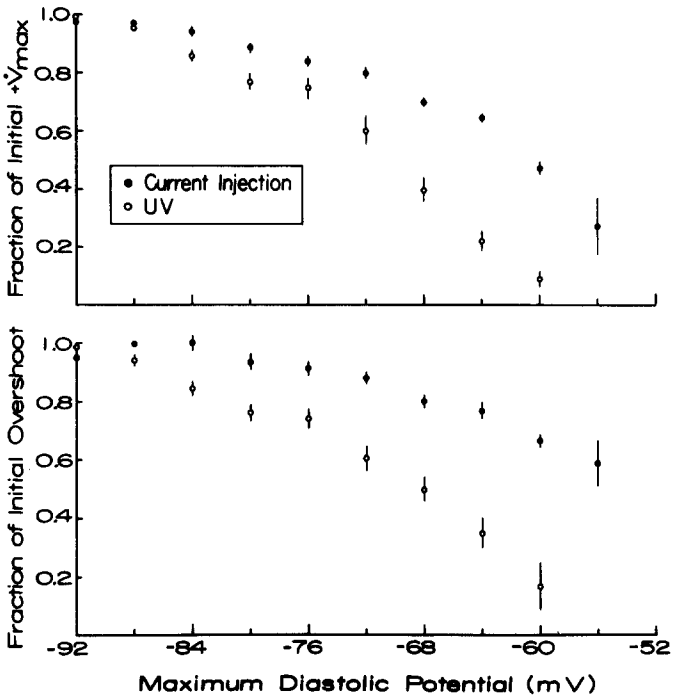

FIGURE 8. Changes in maximum rate of rise (top) and overshoot (bottom) due to depolarization induced by UV at 295 nm (open symbols) and by current injection (closed symbols). Individual values were normalized to 1.0 at the most negative MDP. Each point represents the mean (±SE) for 2–20 action potentials. Values of $+\dot{V}_{max}$ and overshoot were averaged in 4-mV groups from −94 to −54 mV MDP and were plotted at the mean potential of each group.

followed by a rather abrupt cessation of beating. Intracellular recordings during irradiation showed a marked decline in the maximum rate of rise, overshoot, and repolarization phase of the action potential, and a significant change in threshold toward zero. As discussed below, we attribute these results to two effects: a reduction of fast sodium conductance, and depolarization of the heart cell membrane.

The only other investigations of UV-induced effects on muscle preparations with which the present work can be compared are those of Adler (1919) and Azuma and Hill (1926) who showed that involuntary smooth muscle preparations from a variety of sources would contract spontaneously under UV light. Azuma (1927) also described UV-induced repetitive contraction in frog sartorius muscle which increased in rate until reaching a maximum, slowed, and then stopped. More recently Berns et al. (1972) irradiated single mitochondria of embryonic rat ventricular cells with a high power argon laser at wavelengths of 488 and 514 nm. All of these investigators observed changes in the rate of contraction or cessation of contraction depending on the dose, as well as initiation of contraction in quiescent cells. However, none used intracellular record-

ing techniques. Recent studies on nerve axons using voltage clamp techniques (Fox, 1974 *a*; Oxford and Pooler, 1975) may be relevant in analyzing the mechanisms responsible for the termination of beating in the heart cell system. These investigations have revealed a UV-induced decline in membrane sodium conductance and a considerably smaller reduction of potassium conductance which ultimately lead to inexcitability.

The cessation of spontaneous beating which we have observed is most likely the result of a combination of two light-induced effects, a reduction of fast sodium conductance and a depolarization which partially inactivates the sodium system. Both of these processes may be expected to contribute to the marked decline in the maximum rate of rise of the action potential (Fig. 8; Weidmann, 1955; Trautwein, 1973) and to the significant change in threshold illustrated in Figs. 6 and 7. Similar changes limited to threshold and rate of rise were observed during ultraviolet irradiation of nerve axons (Fox, 1972; Oxford, 1974) and were later shown to be due to a direct effect of UV on sodium channels (Fox, 1974 *a*; Oxford and Pooler, 1975). The contribution of a UV-induced reduction of fast sodium conductance to the termination of beating in heart cells is further supported by the following observations: (*a*) The decrease in time to termination of beating with aggregate age (Fig. 5) parallels the increase in TTX sensitivity (presumably attributable to the development of fast sodium channels) between 4 and 7 days (McDonald et al., 1972; DeHaan et al., 1975). (*b*) The action spectrum for the termination of beating is similar to action spectra for the UV-induced reduction of nerve membrane sodium conductance (Fig. 9).

In contrast to the termination of beating, the UV-induced increase in beat rate appears to result from depolarization independent of the effect on the sodium system. Such a depolarization might be expected to increase beat rate either by reducing the magnitude of the potential change required to bring the membrane to threshold, or by increasing the slope of the pacemaker potential (Sperelakis and Lehmkuhl, 1964). In our case the pacemaker potential slope did not change substantially during irradiation, and the beat frequency rose in parallel with the decline in MDP (Fig. 7). In principle, beat rate also could have been altered by a shift of conductance parameters along the voltage axis leading to a change in threshold toward MDP. However, Figs. 6 and 7 indicate the opposite. MDP moved toward threshold while both approached zero potential. Thus, these results support the idea that the reduction in the potential difference between MDP and threshold due to depolarization was the primary cause of the increase in spontaneous rate.

As opposed to our own results with heart cells, UV irradiation of nerve failed to produce significant membrane depolarization (Booth et al., 1950; Lüttgau, 1956; Lieberman, 1967; Fox, 1972; Oxford, 1974). This raises the interesting possibility that muscle and nerve might differ in the mechanisms responsible for this aspect of their response to UV. While the present study is, to our knowledge, the only one to date in which potentials were recorded from a muscle preparation during ultraviolet irradiation, the light-induced initiation of contraction of smooth and skeletal muscle (Adler, 1919; Azuma and Hill, 1926; Azuma, 1927) might be explained on the basis of such a depolarization.

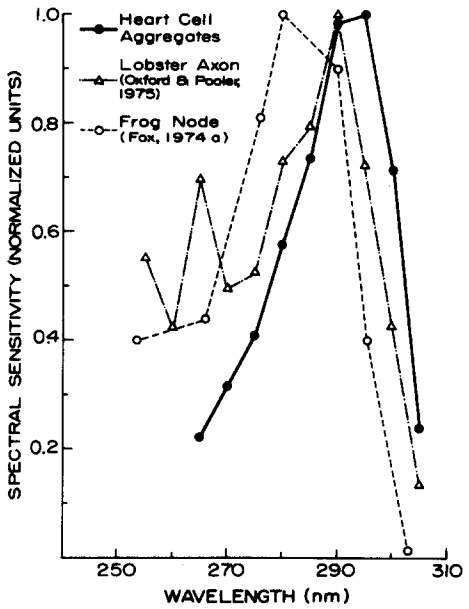

FIGURE 9. Comparison of action spectra of the UV light-induced effect on 7-day heart cell aggregates, lobster giant axons, and frog nodes of Ranvier. The parameters used to calculate these curves were the inverse of the times to stop beating and rate constants for the UV-induced fall in peak sodium current. All three peaks have been normalized to the same maximum and each curve has been corrected for spectral variations in the intensity of light reaching the preparation.

The sensitization of aggregates to UV brought about by a reduction of external potassium concentration was also seen with skeletal muscle (Azuma, 1927) and could be due to a partial depolarization. DeHaan and Gottlieb (1968) found a 7-mV depolarization for a reduction of potassium concentration from 4.2 to 1.3 mM in the case of isolated embryonic heart cells. Calculations of membrane potentials for cardiac Purkinje fibers (Noble, 1965) based on the data of Weidmann (1956) showed about a 25-mV depolarization for a reduction from 2.0 to 1.0 mM. In addition, membrane potentials have been found to influence nerve UV sensitivity. A hyperpolarization of 30 mV reduced the sensitivity of sodium channels by 36% (Fox, 1974 *b*), while depolarization sensitized them to UV (Fox, personal communication).

The depolarization we have observed could result from any of several light-induced effects, including: (*a*) a reduction of membrane potassium conductance, (*b*) inactivation of a hyperpolarizing electrogenic pump (Isenberg and Trautwein, 1974), (*c*) an increase in nonspecific leakage conductance. No substantial changes in leakage conductance were recorded for neural preparations (Fox, 1974 *a*; Oxford and Pooler, 1975); however, in those preparations no depolarization was observed either. To distinguish among the possibilities above, more direct evidence such as that from a voltage clamp analysis is required.

The hypothesis of two independent actions of ultraviolet light on heart cell aggregates may explain the discrepancy between aggregate sensitivity as measured by the inverse of the time to stop beating ($1/t_s$) and the maximum percent increase in beat rate (Fig. 3). From Figs. 7 and 8 and other similar data, aggregates irradiated at 295 nm stop beating at more negative potentials than when depolarized by current injection. Since their rates of firing are determined primarily by the difference between MDP and threshold, aggregates irradiated at 295 nm cease beating before they are allowed to reach their minimum MDP's and therefore their maximum beat rates. At shorter wavelengths where the time to termination of beating is greater, and presumably the effect on the fast sodium system is weak, depolarization dominates. Here aggregates are able to reach their minimum MDP's and maximum attainable rates.

Similar reasoning may apply to the dependence of aggregate sensitivity on age (Fig 5). Although 4-day aggregates may be relatively insensitive to ultraviolet light at 295 nm because they lack a fast sodium conductance mechanism (McDonald et al., 1972), the effect of light-induced depolarization alone may be sufficient to make them inexcitable. At 14 days when aggregates have a well-developed, TTX-sensitive fast sodium system (McDonald et al., 1972) and behave about the same as the intact heart at this stage (Shigenobu and Sperelakis, 1971), they are extremely sensitive to 295-nm light and cease beating before they are able to reach their maximum rates. Thus the curves for aggregate sensitivity measured by ($1/t_s$) and by $(R_{max} - R_o)/R_o$ go in opposite directions.

The differential wavelength dependence of the two measures of aggregate behavior illustrated in Fig. 3 suggests that two different chromophores may be responsible. Proteins exhibit strong absorption between 270 and 300 nm due to the contribution of aromatic amino acids, particularly tyrosine and tryptophan (Wetlaufer, 1962). Since the UV-induced termination of aggregate beating shows a peak sensitivity in this same wavelength region one may suggest that protein absorption initiates this process. Speculation on the nature of the other chromophore is difficult since no peak sensitivity appeared over the wavelength range employed.

A comparison (Fig. 9) of action spectra for the UV-induced termination of beating of 7-day heart cell aggregates and the blockage of sodium channels in the membranes of lobster giant axons (Oxford and Pooler, 1975) and frog nodes of Ranvier (Fox, 1974 *a*) suggests that similar absorbing molecules may be responsible. The relative agreement of the curves for heart cell aggregates and for nerve is good considering that widely differing preparations were employed and different parameters were used to quantify the effects. Furthermore, these results are consistent with earlier action spectra based upon measurements of increases in action potential threshold for frog nodes of Ranvier (Booth et al., 1950) and measurements of the total energy to block action potential conduction in crab axons (Lieberman, 1967). The small differences which do exist could be distortions resulting from shielding by other cells (Fig. 4) or by other components of the membrane. This question could possibly be resolved by determining an absorption spectrum for isolated myocytes.

In summary, we have attributed the observed UV-induced increase in beat rate and termination of beating of heart cell aggregates to separate complementary processes, a depolarization of the membrane and a decline in fast sodium conductance. Each of these effects may derive from a different photon-substrate interaction. Final conclusions as to the specific membrane processes involved must await a voltage clamp analysis of membrane ionic currents.

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