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SHORT COMMUNICATIONS

Argon Laser Micro-irradiation of Mitochondria in Rat Myocardial Cells in Tissue Culture

III. Irradiation of Multicellular Groups

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M. W. BERNS, D. C. L. GROSS AND W. CHENG. Argon Laser Micro-irradiation of Mitochondria in Rat Myocardial Cells. III. Irradiation of Multicellular Groups. *Journal of Molecular and Cellular Cardiology* (1972) **4**, 427–433. Single mitochondria of contracting myocardial cells were irradiated with an argon laser microbeam and the contractility changes of the cells were noted. Four series of irradiation experiments were conducted: (i) one cell of a two-cell group was killed, (ii) one cell of a multicell group was killed, (iii) one cell of a two-cell group was damaged, (iv) one cell of a multicell group was damaged. In series (i) and (ii) a majority of cells first fibrillated and then either returned to rhythmic contraction rate, or stopped contracting. In series (iii) and (iv) the individual contractility pattern following irradiation was quite variable, but by 30 min post irradiation a majority of the cells had returned to a rhythmic contraction rate. These studies demonstrate the feasibility of using the laser microbeam to investigate cell–cell interactions, and factors involved in regulating myocardial cell contractility.

1. Introduction

The ability to alter the contractility of individual myocardial cells by *in vitro* laser microbeam irradiation of single mitochondria has been demonstrated [*2*, *3*, *6*]. Correlations have been made between mitochondrial phase density, lesion morphology, laser energy density, and contractile response. Cellular responses varied from cell death, to no change in contraction. Many cells were observed to enter into "fibrillation" (rapid, uncoordinated contractions) and then return to a rhythmic contraction rate often identical to the pre-irradiation rate. Some cells stopped contracting after irradiation and then returned to a rhythmic contraction rate. Still other cells stopped contracting and remained in that state for an indefinite time period.

Since the initial studies were designed to define the functional response associated with a particular laser energy, mitochondrial phase density, and lesion morphology, care was taken to irradiate only single cells. This was necessary because a myo-

cardial cell can influence the contractility rate of another myocardial cell by direct physical contact, or by contact through an intermediate non-myocardial cell [*4*, *5*]. De Haan [*4*] has demonstrated that in a population of cultured myocardial cells, isolated single cells may contract at various rates. However, when these cells come into contact with each other both cells contract in synchrony, often at the rate of the faster cell. In addition there are myocardial cells (non-pacemaker cells) that normally are not able to initiate their own contractions, but when they contact other contracting cells (pacemaker cells), they are induced to contract in synchrony with the pacemaker cells. Both of these phenomena (the faster cell speeding up the slower cells, and the initiation of contraction of a non-pacemaker cell) appear to involve electrophysiological interactions via cell membranes.

The preceding observations suggested that perhaps by selective micro-irradiation of one myocardial cell, specific and predictable response could be elicited in the adjacent myocardial cells. Such an approach might be very useful in the study of cell–cell interactions, and in particular the inter and intracellular mechanisms involved in regulating myocardial cell contractility.

Based upon the responses of laser micro-irradiation of single myocardial cells [*2*, *3*] a series of investigations was undertaken to determine (*i*) if cells in contact with the irradiated cell would undergo contractility changes and (*ii*) to what extent predictable responses could be elicited.

2. Materials and Methods

Myocardial cells were established in Rose culture chambers according to the method of Mark & Strasser [*5*]. The ventricles of 1 to 4-day-old rats were minced, subjected to stepwise trypsinization, and suspended in minimal essential Eagles medium with essential and non-essential amino acids, and 10% fetal calf serum. Medium was changed every 2 to 4 days, and cells were maintained at 37°C and pH of 7.4.

The laser microbeam was the one described in detail elsewhere [*6*]. A mixed wavelength beam (488 nm, and 514 nm) from a pulsed argon laser was directed into a Zeiss photomicroscope and focused to a micro-spot 0.5 to 1.0 micrometer. Energy density in the focused spot was 20 to 50 μJ, and the duration of the laser pulse was 50 μs. Irradiation was accomplished by locating a contracting myocardial cell under the microscope and viewing it on a television monitor. By moving the cell under cross hairs on the television monitor denoting the focal point of the laser, specific regions of the cell could be irradiated. The damaged cell always had a large, dark mitochondrion irradiated. Contractility changes in the irradiated and adjacent cells were noted at the time of irradiation, 3 to 5 min and 30 min post-irradiation. Four types of experiments were performed: (i) two adjacent touching cells were selected, one cell was killed with a severe lesion and contractility changes noted in the non-irradiated cell; (ii) a group of more than two myocardial cells was selected, and one cell in the group was killed, contractility changes were noted in the other cells; (iii) two adjacent cells were chosen; one cell was irradiated,

and damaged, but not killed; contractility changes in both cells were noted; (iv) a group of more than two myocardial cells was selected, one cell was damaged and contractility changes in all cells were noted.

3. Results

Two cells: kill one, observe other

A total of nine groups of two cells each were irradiated. Prior to irradiation it was determined that the two cells were not in physical contact with any other contracting cells in the chamber, and that both of the cells were contracting rhythmically and in synchrony. Pre- and post-irradiation beat rates, and the contractility sequences of the unirradiated surviving cells are presented in Table 1 (cells no. 1 to 9).

TABLE 1. Contractility changes in surviving cells: one cell in group killed.

Cell group number	Number of cells in group	Pre-irradiation rate (beats/min)	0 min*	3 min	30 min
1	2	44	Fibrillation	Fibrillation	No contraction
2	2	52	No contraction	Fibrillation	60†
3	2	92	No contraction	Fibrillation	96
4	2	52	52	52	52
5	2	48	No contraction	Fibrillation	No contraction
6	2	32	No contraction	Fibrillation	28
7	2	48	No contraction	Fibrillation	No contraction
8	2	32	No contraction	Fibrillation	28
9	2	28	No contraction	Fibrillation	28
10	3	103	No contraction	Fibrillation	No contraction
11	3	30	No contraction	Fibrillation	100
12	4	77	Fibrillation	70	70
13	3	Rhythmic (rate?)‡	No contraction	Fibrillation	No contraction
14	3	60	180	180	180
15	3	72	No contraction	Fibrillation	87
16	3	192	Fibrillation	210	210
17	8	138	Fibrillation	Fibrillation	Fibrillation
18	5	100	Fibrillation	Fibrillation	Fibrillation

* Immediately following irradiation.
† Beats/minute.
‡ Pre-irradiation beat rate not recorded.

Of the nine groups, all but no. 4 demonstrated fibrillation in the surviving non-irradiated cell. Of the eight cells that underwent fibrillation, seven ceased contracting prior to fibrillation, immediately after irradiation of the killed partner. Two of the cells (no. 5 and no. 7) did not resume contracting after fibrillation, and the five

cells that did resume contracting, contracted at rates very close to the pre-irradiation beat frequency by 30 min post-irradiation. The surviving cell in group no. 1 fibrillated immediately after its partner was killed, and by 30 min post-irradiation had ceased all contractile activity.

Group of cells: kill one, observe others

A total of nine groups with three or more contracting cells were chosen; only one cell in the group was irradiated and killed. All cells in the groups were contracting rhythmically and at the same beat frequencies prior to irradiation. All cells in each group responded the same way post-irradiation. The data are summarized in Table 1 (cells nos 10 to 18).

Fibrillation occured in eight of the nine cell groups. Two of the cell groups (nos 11 and 15) stopped contracting, fibrillated, and returned to a rhythmic contracting rate by 30 min. Two other cell groups (nos 12 and 16) fibrillated immediately after irradiation and returned to a rhythmic rate by 3 min post-irradiation. Cell groups no. 17 and no. 18 fibrillated immediately after irradiation and continued fibrillating for 30 min. The only group that did not fibrillate (no. 14) sped up its contraction rate from 60 to 180 beats/min.

Two cells: damage one, observe other

In this series of experiments seven groups of two rhythmically contracting cells were chosen, and one cell per group was irradiated with a sub-lethal dose of energy. Contractility changes of both cells are summarized in Table 2 (cells nos 19 to 26).

In four cell groups (nos 19 to 22) both cells in the group responded identically. In the other three groups (nos 23 to 25) the two cells initially responded differently but by 3 min post-irradiation were behaving similarly. Four of the seven cell groups (nos 21, 23 to 25) returned to rhythmic contractions following an initial alteration in contractility (either fibrillation or cessation of contraction) and two cell groups (nos 19 and 20) stopped all contraction by 30 min post irradiation. One cell group (no. 22) sped up its contraction rate (72 beats/min to 116 beats/min) immediately following irradiation and remained at the increased rate for 30 min. In five of the seven groups, either one or both cells fibrillated during the post-irradiation phase.

Group of cells: damage one, observe others

Groups of three to six contracting cells were selected for irradiation. One cell per group was irradiated with a sub-lethal laser energy density and the contractility changes of the irradiated cell and the other non-irradiated cells were noted (Table 2, cells nos 26 to 36).

Of the 11 cell groups irradiated, three did not exhibit any contractility changes, even in the irradiated cell (nos 28, 33, 35). Seven of the eight cell groups that did undergo contractility changes, returned to rhythmic contractions by 30 min post-

TABLE 2. Contractility changes in surviving cells: one cell in group damaged

Cell group number	Number of cells in group	Pre-irradiation rate* (beats/min)	0 min	3 min	30 min
19	2	68	Both cells no contraction	Fibrillate	No contraction
20	2	80	Both cells no contraction	Irregular†	No contraction
21	2	16	Both cells fibrillate	No contraction	16
22	2	72	Both cells 116	116	116
23	2	152	Irrad. cell no contraction;	Fibrillate	150
			Non-irrad. cell fibrillate	Fibrillate	150
24	2	108	Irrad. cell fibrillate;	116	116
			Non-irrad. cell No contraction	116	116
25	2	88	Irrad. cell no contraction;	84	84
			Non-irrad. cell Fibrillate	84	84
26	6	56	Irrad. cell no contraction; Other cells, no effect (78)‡	78	78
27	3	64	No contraction	64	64
28	6	60	60	60	60
29	3	52	Irregular	52	52
30	5	132	Fibrillate	No contraction	180
31	6	44	No contraction	Fibrillate	44
32	4	92	Fibrillate	No contraction	0
33	3	132	132	132	132
34	3	140	Irrad. cell fibrillate	140	140
			Other cells no contraction	140	140
35	3	176	176	176	176
36	3	60	Fibrillate	60	60

* All cells have same pre-irradiation beat rate.

† These cells exhibited normal contractions of irregular periodicity.

‡ No effect on rhythmicity, but there did appear to be an increase in beat frequency.

irradiation and one cell group (no. 32) was not contracting at all by 30 min post-irradiation. Fibrillation was observed in five of the eight cell groups. In two of the cell groups (nos 26, 34) the irradiated cell initially behaved differently from the rest of the cells in the group, but by 30 min post-irradiation all cells were contracting rhythmically at the same rate.

4. Discussion

There does not seem to be any doubt that cells in contact with the irradiated cell can be induced to undergo contractility changes. Of the 36 cell groups irradiated, only four did not demonstrate alterations in contractility to all the cells in the group. Three of the four were in the last irradiation series ("Group of cells: damage one, observe others").

It is less easy to delineate consistent and predictable contractility responses. The most consistent patterns were in the cell groups where one of the cells was killed by the irradiation (see first two "Results" subsections). In 61% (11/18) of these groups, the unirradiated cells in each group stopped contracting immediately after irradiation, and fibrillated by 3 min post-irradiation. Some of these cells returned to a rhythmic contraction rate, and others stopped contracting entirely. It is possible that in the latter cases, the killed cell was the only pacemaker cell in the group. This might explain why the cells did not resume contracting. If the myocardial cell population contains both pacemaker and non-pacemaker cells [*4*, *5*] it would seem likely that in the case of two-cell groups, associations would be formed between two pacemaker cells, or a pacemaker and non-pacemaker cell (since we only chose contracting cells, the association of two non-pacemaker cells could be neglected). By killing one cell of the pair, the remaining cell could be either a pacemaker or non-pacemaker. In the latter situation the surviving cell would not be contracting, and in the former it would. Recording of the single cell membrane potentials with micro-electrodes will resolve this question.

The least consistent contractility changes were observed in the cell groups where the irradiated cell was damaged but not killed (see second two "Results" subsections). The patterns of contractility changes prior to the final contractility state (30 min post-irradiation) were unpredictable. Indeed, the irradiated cell often initially contracted differently from the rest of the cells in the group. However, in 67% (12/18) of these groups all the cells had returned to a rhythmic contraction rate by 30 min post-irradiation. This compares to 55% (10/18) for the other two experimental groups (see first two "Results" subsections).

Perhaps the two most frequent responses observed in all the experimental groups were fibrillation and a return to rhythmic contractility. Fibrillation was observed in 70% (25/36) of the experimental cell groups, and a return to rhythmic contractility was noted in 61% (22/36) of the cell groups. These figures compare rather favourably to those previously reported for contractility changes in single

irradiated cells [2]: fibrillation in 82% of the cells, and return to rhythmic contractions in 82% of the cells. The fact that the percentages reported for multiple cell groups are lower is not surprising since the interactions between cells must be great compared to the situation of a single isolated cell. The mechanisms by which the cells communicate with each other, and indeed, the reasons for the high percentage of induced fibrillation and the return to a rhythmic contraction rate, is still unresolved. However, electrophysiological and biochemical methods should permit elucidation of these phenomena. The ability to induce these responses in groups of cells will make application of these techniques more feasible.

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