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Interferon Inhibits Cardiac Cell Function *in Vitro* (41043)

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Abstract. Interferon which has been shown to exert important effects on cellular function was utilized to investigate its effect on cardiac cell beating *in vitro*. When steadily pulsating rat cardiac cultures were continuously exposed to rat interferon for 24 hr, a decrease in the beating rate was observed. Mouse interferon which also exerted antiviral activity on rat heart nonmuscle cells also decreased the beating rate of rat cardiac cultures. Human leukocyte interferon when tested at the same dose at which rat interferon was active, exhibited no antiviral activity in rat heart nonmuscle cells and did not exert beating rate effects. When mouse interferon was incubated with antiserum prepared against mouse interferon both antiviral and beating activity were neutralized to the same extent. None of the interferons used produced morphological effects on the heart cells and with rat interferon the beating rate effect was reversible. This finding, that interferon affects cardiac cell function *in vitro*, may have relevance to clinical application.

Interferon exerts important effects on cellular functions apart from its inhibitory effect on cell division (1). Modification of cellular functions has been demonstrated by: either inhibition or enhancement of the synthesis of specific inducible cell products (2–4); enhancement of function of specialized cells, such as cytotoxicity of sensitized T lymphocytes (5) or natural killer cells (6), phagocytosis by macrophages (7), and excitability of cultured neurons (8); or inhibition of cell motility (9). Cardiac cells growing in culture maintain their *in vivo* ability to beat spontaneously. Their consistent autorhythmic contractions *in vitro* provide an excellent model for assaying cellular function. The post mitotic nature of cardiac cells eliminates the complication of cell proliferation on functional assays in dividing cell systems. In view of the broader application interferon may have in the future as an anticancer agent, our results, which show that interferon significantly reduces the beating rate of cardiac cells in culture, may have some influence on clinical usage.

Materials and Methods. *Cells and beating rate recording.* Cultures of myocardial cells from newborn Sprague–Dawley rats were prepared as previously described (10). Single cell suspension of 3×10^5 cells/ml were seeded by placing 0.1-ml droplets in the center of 35-mm (Costar) culture dishes. The droplets were incubated overnight and the following morning each culture received 2 ml of fresh minimal essential medium (MEM) supplemented with 10% fetal bovine serum. The medium was changed every 48 hr, to ensure consistent beating rates, and interferon treatments did not begin until cells were in culture for at least 7 days. It has previously been shown by electron microscopy that heart cells require several days in culture before they fully recover from trypsinization (11). At the time of treatment each culture pulsates rhythmically as a single unit. Thus the rate determined in one area of the culture dish is representative of the entire culture's pattern of beating. In this way cultures may be returned to the microscopic stage at intervals without the need to fix the exact location of cell area of each reading. A microscope stage has been designed to accurately control temperature ± 0.05° and pH ± 0.2, two variables to which cardiac cell function is particularly sensitive (12). An electronic system has been developed which quantitatively records beating patterns. The system consists of a television camera

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(RCA Model TC1005) which transmits the microscopic image of the cells onto a television monitor (Sanyo, Model VM 4155); a photodiode which can be manually positioned to any point on the monitor screen, detects changes in light intensivity as the cells beat. Each beat is permanently documented by a polygraph recorder (Grass, Model 7) which is connected to the photodiode device. The difference in amplitudes from one recording to another reflects the contrast and total movement of the selected area on the screen and is not used in these experiments as a measure of contractility.

Interferon preparations. Rat interferon was prepared from Wistar rat embryo fibroblasts inoculated with Newcastle disease virus (NDV), concentrated and partially purified as previously described (13). The interferon titer of these preparations was 2.5×10^{-5} when assayed on nonmuscle cells (a mixture of fibroblasts and epithelial cells) isolated from the heart cell cultures. The protein content of the interferon and mock interferon prepared in a manner identical to that used in the preparation of interferon but without the inducer was approximately 2.0 mg/ml.

Mouse interferon was prepared from Swiss mouse C-243 cells induced with NDV, concentrated and partially purified as previously described (14). The specific activity of these preparations varied between 0.3 and 1.7×10^{7} reference units/mg of protein and the protein content varied between 1.5 and 3.4 mg/ml. These preparations had a titer of 1.2×10^{-6} as assayed on mouse L cells and exhibited antiviral activity on rat heart nonmuscle cells. The ratio of heterologous activity to homologous activity was 1:40.

Partially purified human interferon (specific activity 1.5×10^{6} units/mg of protein) was prepared in leukocyte suspension inoculated with Sendaï virus and was generously provided by Dr. K. Cantell (15). This interferon preparation had a titer of 1.2×10^{6} as assayed on human skin fibroblasts.

All interferon preparations were assayed by inhibition of cytopathic effect in cells cultivated in 0.2-ml volumes in Falcon microtiter II plates and challenged with 100 $TCID_{50}$ of vesicular stomatitis virus (VSV) as previously described (16).

Mouse anti-interferon serum. Mouse antiinterferon serum, prepared by repeated inoculation of sheep with partially purified C-243 cell interferon, was generously provided by Dr. I. Gresser (17). A 1:2,000,000 dilution of serum neutralized 8 units of mouse interferon as assayed on L cells inoculated with VSV.

Results. When rat interferon was added to the medium of steadily pulsating cardiac cultures, a decrease in the beating rate was observed (Fig. 1). The effect was related to the dose of rat interferon employed and was still significant when 640 units of rat interferon was added (Fig. 2).

The beating rate effect was first detected 24 hr after the addition of interferon and became more pronounced after 48 hr. Further exposure to interferon, however, did not significantly increase its effect on the beating rate (Fig. 3). The treated cultures maintained both the slower beating rate and rhythmicity for several days. When interferon was removed from cultures treated for 7 days, the cells regained their initial beating rate within 24 hr (data not shown). Both mock rat cell interferon and MEM, were used as controls. When either was added to the cultures no effects on beating rates were seen (Fig. 3).

Further, when cultures were fixed in gluteraldehyde, osmium, taken through ascending steps of alcohol dehydration, embedded in epon, sectioned, and stained, no morphologic differences could be detected between treated and untreated cells.

In order to confirm that the observed effects on cardiac cell beating were indeed due to interferon, preparations from different species of animal were utilized. We found that only rat and mouse interferon which exerted antiviral activity on rat heart nonmuscle cells had an effect on the beating rate. In the experiment presented in Fig. 4, the cross activity of mouse interferon on heart nonomuscle cells was one-fortieth. Under these conditions, approximately 400 "effective" rat units of mouse interferon exerted a significant effect on the beating rate. Furthermore, treatment with antiserum prepared against mouse interferon to-

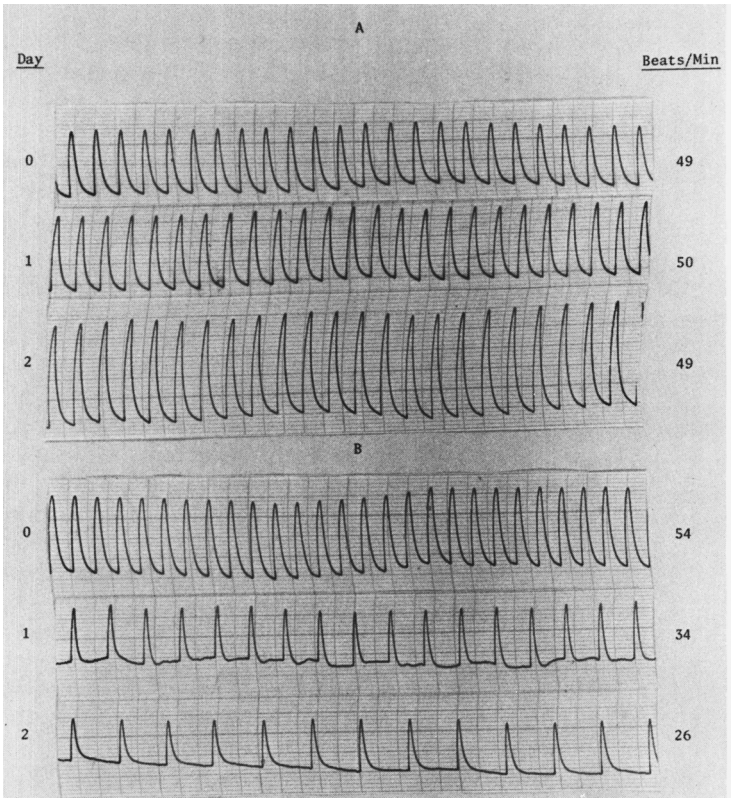

FIG. 1. Effect of rat interferon on the beating rate of rat cardiac cells in culture. Upper recordings (A) are beats/min at 5 mm/sec (polygraph speed) for an untreated culture at the indicated days. Lower recordings (B) are beats/min at the same polygraph speed for culture continuously treated with 1.28×10^4 units of rat interferon. Recording on Day 0 is just before the addition of interferon.

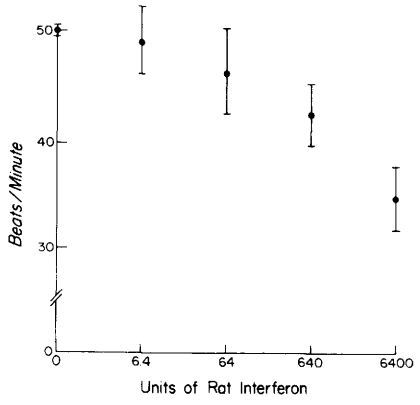

FIG. 2. Dose-related effect of rat interferon on the beating rate of rat cardiac cells in culture. One-minute determinations were recorded 24 hr after the addition of interferon at the indicated concentrations. Each point is the average beating rate of three separate cultures and standard deviations are as indicated.

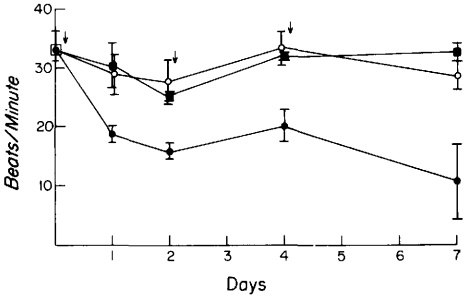

FIG. 3. Long-term effect of rat interferon on the beating rate of cardiac cells in culture. One-minute recordings were made before the change of medium which occurred at Days 0, 2, and 4. Rat interferon 1.28×10^4 units (●), mock preparation (○), and MEM medium (■) were added at the indicated times (↕). Day 0 point (◙) represents the average beating rate of all nine cultures. Each point is the average beating rate of three separate cultures and standard deviations are as indicated.

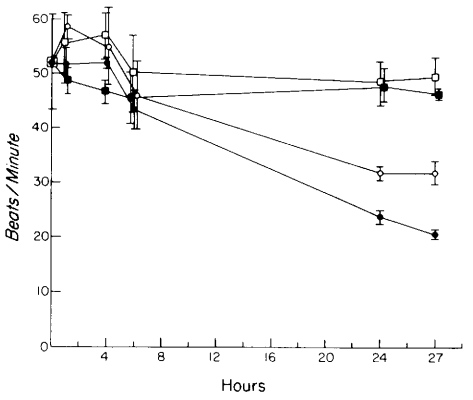

FIG. 4. Effect of homologous and heterologous interferon on the beating rate of rat cardiac cells in culture. Cardiac cells were treated with 1.28×10^4 units of rat interferon (●), 1.6×10^4 units of mouse interferon (○), 1.6×10^4 units of human leukocyte interferon (□), and MEM medium (■), immediately after initial reading ◘ (average beating rates of 12 cultures). Note that at 6 hr of treatment no discernible effect could be detected. Each point is the average beating rate of three separate cultures and standard deviations are as indicated.

tally abolished the inhibitory activity of mouse interferon on cardiac cell beating (i.e., after cells were treated for 24 hr the average beating rate/min for three cultures/group was as follows: antiserum-treated cultures (control), 90.0 ± 11.3; mouse interferon-treated cultures, 60.7 ± 10.1; and antiserum incubated with mouse interferon, 87.0 ± 2.6; the average beating rate/min of the nine cultures before treatment was 86.4 ± 9.2); the antiserum neutralized both antiviral and beating inhibitory activity to the same extent. When partially purified human leukocyte interferon was tested at the same dose at which rat interferon was active, it exhibited no antiviral activity in rat heart nonmuscle cells and did not exert beating rate effects (Fig. 4).

Discussion. Our findings broaden the spectrum of activity of interferon on cellular function. At the present time the mechanism for the action of interferon on cardiac cell function remains unknown. Effects which are suggestive of cell surface modification are usually observed shortly after addition of interferon (<12 hr) (8, 18). Since our effects were detected later (24 hr), and the synchrony and rhythmicity of beating which depends on intact functional membranes are unperturbed, direct modification or damage of cell membranes seems unlikely. It remains possible, however, that interaction of interferon at the cell membrane leads to subsequent metabolic effects. Harary *et al.* (19) have used several metabolic inhibitors to demonstrate the sensitivity of cardiac cell beating rate to intracellular ATP levels. They demonstrated that for short periods of time (30 min) heart muscle cells could undergo loss of either glycolysis or oxidative phosphorylation without significant drops in ATP levels and with no effect on beating rates. However, if both pathways are inhibited at the same time, ATP levels drop significantly and beating rates decrease. Thus the possibility exists that ATP levels are significantly altered in interferon-treated cells. We are currently investigating this possibility.

Recently Blalock and Stanton (20) have reported an increase in the beating frequency of mouse myocardial cells 2 min after the addition of mouse interferon. Their experiments differ from ours in that they began drug treatment 24 hr after cells had been trypsinized and seeded whereas we allowed the cells to grow in culture for 7 days before initiating interferon treatments. It has previously been shown by several different investigators (21, 22) that chick, mouse, and rat cardiac cells are severely damaged after trypsinization with marked disorganization of myofilaments and complete loss of Z line material and require nearly 7 days in culture to regain a morphology comparable to undissociated tissue (11). It has also been demonstrated, that trypsin persists on the cell surface for as long as 24 hr after application (23) and enzyme activity can be demonstrated intracellularly for up to nearly 48 hr (24). Under these circumstances it is difficult to interpret effects of agents which act within 2 min on the function of newly trypsinized cardiac cells. Since the short-term effects of interferon reported by the above authors were detected at room temperature and high pH, it is possible that under these conditions interferon acts differently on cardiac cell beating than at physiologic pH and temperature.

Our findings clearly show an inhibitory effect of interferon on cardiac cell function *in vitro*. It may be that interferon also affects cardiac function *in vivo* and in view of the clinical trials of interferon currently in progress, this possibility should be investigated.

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