Pulsing Electromagnetic Fields Induce Cellular Transcription

Abstract. *Weak, pulsing electromagnetic fields can modify biological processes. The hypothesis that responses to such induced currents depend on pulse characteristics was evaluated by using transcription as the target process. Two pulses in clinical use, the repetitive single pulse and the repetitive pulse train, were tested. These pulses produced different results from each other and from controls when transcription in dipteran salivary gland cells was monitored with tritiated uridine in transcription autoradiography, cytological nick translation, and analysis of isolated RNA fractions. The single pulse increased the specific activity of messenger RNA after IS and 45 minutes of exposure. The pulse train increased specific activity only after 45 minutes of exposure.*

Basic cellular phenomena such as growth, differentiation, dedifferentiation, and repair have been modified by weak direct current (I). Electrical currents induced in tissues by weak, pulsing electromagnetic fields (PEMF's) are also biologically active in regeneration and repair (2). Recently, the repetitive pulse train (PT) has been shown to have a significant clinical impact in repairing recalcitrant bone fractures (3), while the repetitive single pulse (SP) has had beneficial effects in avascular necrosis and in osteoporosis (4). These different effects appear to depend on specific waveform parameters in the driving pulse and in the asymmetrical induced pulsing current, parameters that may be determined in part by passive electrical characteristics of the target tissue.

We tested the hypothesis that exogenous PEMF's trigger selected cellular responses *(5)* by studying alterations in normal RNA transcription patterns in the salivary gland chromosomes of the dipteran *Sciara coprophila*. The nuclei of the nondividing cells of the salivary gland contain four polytene chromosomes engaged in interphase synthetic functions, including RNA transcription and cyclical DNA replication (6). Normal transcription patterns during cellular differentiation in salivary gland chromosomes of this organism are known from transcription autoradiography. Thus, transcription can be followed at the cytological level and correlated with chromosome structure by studying banding or puff formation in the giant chromosomes (6).

Salivary glands of late fourth-instar female larvae were dissected in Schneider's *Drosophila* medium containing [3H]uridine (250 uCi/ml; 40.8 Ci/mmole) (New England Nuclear). Whole glands (attached to the larval bodies) in medium were placed in PEMF's for various periods (5 to 90 minutes). Controls for these experiments were established under conditions that were identical except for the absence of the fields. The waveform and other characteristics of the fields are shown in Fig. 1.

Transcription was measured in three ways. First, nascent RNA chains attached to specific chromosome regions were identified by conventional autoradiography. Second, regions of the chromosomes sensitive to deoxyribonuclease I were examined by nick translation (with [125I]deoxycytidine

triphosphate) directly on the DNA of the cytological preparations (7). Deoxyribonuclease I preferentially attacks transcriptively active regions of chromatin (8). Autoradiographic patterns resulting from either approach are similar, but the use of nick translation produces greater specificity in the labeling pattern. Third, RNA's of various size classes were isolated by sucrose density gradient centrifugation and analyzed for changes in the pattern of [3H]uridine incorporation. These tests showed characteristic alterations of transcription by each of the pulse patterns. Various size classes of RNA were influenced by both waveforms in a different manner. Furthermore, the responses to different periods of exposure were different for the two pulse types.

The transcription autoradiogram of a cell incubated in [3H]uridine in the presence
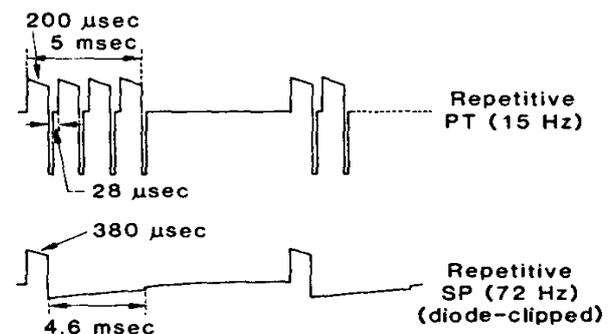

Fig. 1. Major waveform characteristics of the SP and PT stimuli (Biosteogen system 204, Electro-Biology, Inc.). Pulse amplitude, on a calibrated coil probe of IS mV (coupled to a Tectronix 5103N oscilloscope), was equal to 1.5 mV per centimeter of cortical bone (2). The glands were exposed to PEMF's in 0.5 ml of Schneider's *Drosophila* medium in petri dishes (60 by IS mm) between a pair of 10 by 10 cm Helmholtz aiding coils, delivering an average magnetic field parallel to the floor of the dish. Coil orientation was vertical. The rate of change of the magnetic field *(dB/dt)* was approximately 0. l G/usec for PT's and 0.05 G/ usec for SP's (IS). Frequency content of the two pulses, derived by discrete Fourier transforms, differs significantly (16).

of the SP field for 45 minutes are compared to a control autoradiogram in Fig. 2, A and B. The pattern of incorporation seen after 15 minutes of exposure was almost identical to that observed after 45 minutes. At IS and 45 minutes this pulse produced a marked and specific increase in RNA transcription in most of the bands and interbands of the chromosomes. At 30 minutes transcription was low (approximately equivalent to that seen in the controls). Thus a bimodal response occurred with respect to time in the SP field. Figure 2C shows a nicktranslated cytological preparation from glands stimulated for 45 minutes with SP's. Compared to the transcription autoradiograms, more specific "hot spots" can be seen in the nick-translated chromosome preparation. The results of placing the cells in the PT field for 45 minutes are compared to control results in Fig. 2, D to F. This pulse resulted in a gradual increase in transcription up to 45 minutes. Even after 45 minutes of stimulation, transcription autoradiograms of chromosomes exposed to the PT field showed much lighter labeling than autoradiograms of SP-exposed chromosomes. They were, however, labeled more heavily than the control preparations. After nick translation some specifically active regions were detected; they were most prominent after 45 minutes of exposure to either field (Fig. 2, C and E). Control preparations (not shown) were indistinguishable from the controls shown in Fig. 2, B and

E. Effects of both pulses declined, however, after 60 minutes of continuous exposure.

Direct sucrose gradient analysis of RNA confirmed the results of the transcription autoradiography. Although a fourfold increase in total RNA was seen after 15 and 45 minutes of salivary gland exposure to the SP field, the messenger RNA (mRNA) size class increased 11fold (Fig. 3A). At 45 minutes mRNA exceeded the control values 13-fold (Fig. 3B). In cells treated with PT's, all RNA size classes were at control levels after 15 minutes; thereafter there was a linear increase in size classes up to 45 minutes. After 45 minutes all RNA size classes had increased (Fig. 3B).

Time-dependent induction of RNA synthesis by exposure to PEMF's has been detected by three independent methods. Different pulse characteristics, however, induce different qualitative and quantitative responses in the patterns of RNA synthesis. There are distinct differences in the pattern of RNA transcription induced by PEMF's as compared with other experimental stress conditions, such as heat shock or premature administration of ecdysone (6, 9). For example, the most notable response of Sciara salivary gland chromosomes to heat shock is an unusually marked increase in the transcription of ribosomal RNA (9). In ecdysone-treated cells, transcription patterns are confined to regions
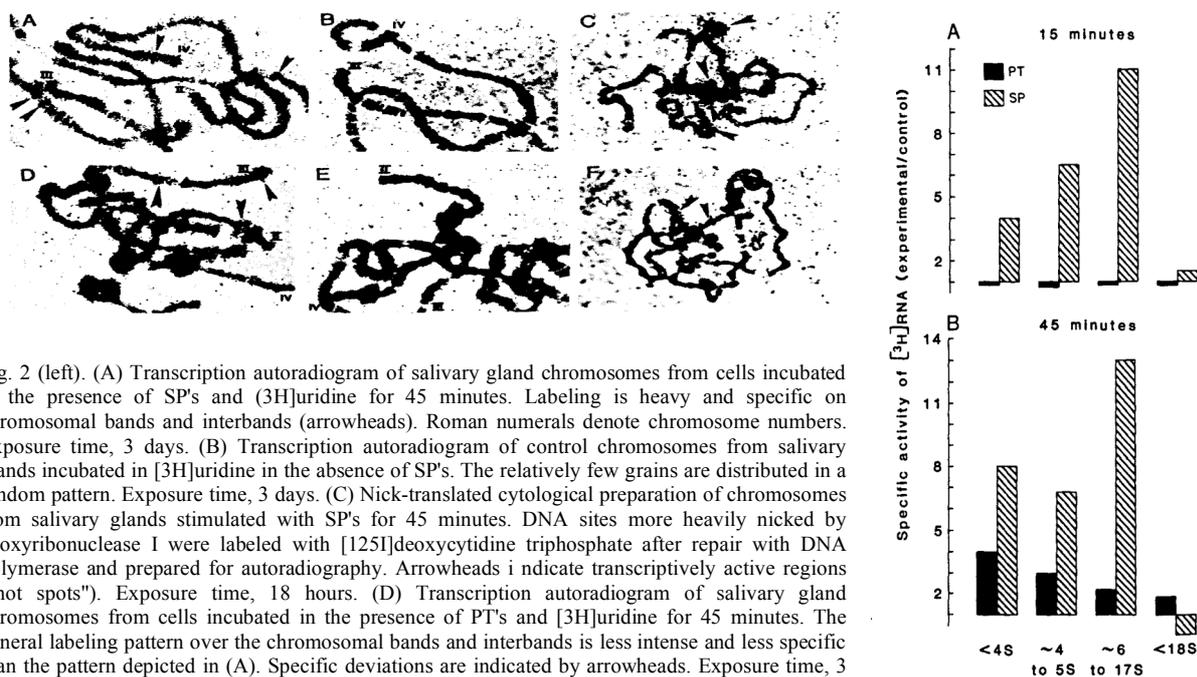

Fig. 2 (left). (A) Transcription autoradiogram of salivary gland chromosomes from cells incubated in the presence of SP's and (3H)uridine for 45 minutes. Labeling is heavy and specific on chromosomal bands and interbands (arrowheads). Roman numerals denote chromosome numbers. Exposure time, 3 days. (B) Transcription autoradiogram of control chromosomes from salivary glands incubated in [3H]uridine in the absence of SP's. The relatively few grains are distributed in a random pattern. Exposure time, 3 days. (C) Nick-translated cytological preparation of chromosomes from salivary glands stimulated with SP's for 45 minutes. DNA sites more heavily nicked by deoxyribonuclease I were labeled with [125I]deoxycytidine triphosphate after repair with DNA polymerase and prepared for autoradiography. Arrowheads i ndicate transcriptively active regions ("hot spots"). Exposure time, 18 hours. (D) Transcription autoradiogram of salivary gland chromosomes from cells incubated in the presence of PT's and [3H]uridine for 45 minutes. The general labeling pattern over the chromosomal bands and interbands is less intense and less specific than the pattern depicted in (A). Specific deviations are indicated by arrowheads. Exposure time, 3 days. (E) Transcription autoradiogram of control chromosomes from salivary glands incubated in [3H]uridine in the absence of PT's. Exposure time, 3 days. (F) Nick-translated cytological preparation of chromosomes from glands stimulated with PT's for 45 minutes. DNA was labeled with [125I]deoxycytidine triphosphate and prepared for autoradiography. Hot spots are denoted by arrowheads. Exposure time, 18 hours. (All magnifications x 1240). Fig. 3 (right). Tritiated RNA isolated after exposure to SP's or PT's (1 7). Approximately 100 salivary glands (2 x 103 cells) were used for each set of time points (four experiments each). Gradients of 5 to 30 percent sucrose were run for 17 hours at 32,000 rev/min in an IEC ultracentrifuge. The gradients were collected with an automatic fraction collector to monitor optical density. A portion was removed from each fraction and its radioactive pattern was determined. The only major difference in the radioactive pattern (compared to the profile of optical density) consisted of highly radioactive fractions of less than 4S, probably reflecting partially transcribed RNA. Fractions of each size class were combined on the basis of these profiles and specific activity was determined and compared to RNA of unexposed cells run in parallel gradients. *Analysis of SP's:* Specific activity of RNA in experimental samples, 10,000 to 15,000 dpm/ug. Specific activity of RNA in controls, 2000 to 4000 dpm/ug. Approximately 50 ug of the experimental and control samples was used for each gradient. Size classes greater than 18S were combined since relative incorporation was not significant when compared to controls. *Analysis of PTs:* Specific activity of RNA in experimental samples, 8000 to 10,000 dpm/ug. Specific activity of RNA in controls, 2000 to 4000 dpm/ug. About 50 ug of the experimental and control samples was used for each gradient.

of the salivary chromosomes that will eventually form puffs (6). Neither of these effects has been observed in cytological preparations after PEMF induction. Other types of pulses have been shown to initiate the uncoiling of DNA (I0, 11).

It is appropriate to consider whether these findings can be correlated with the results of clinical studies in which the skeletal system was exposed to PEMF's. If transcription is affected by the availability of Ca", as was suggested by our preliminary findings (12), this may affect the mode of SP and PT action both molecularly and clinically. Pulse trains are used clinically to elevate cellular calcium and to trigger calcification of fibrocartilage in disunited fractures (3). Single pulses, on the other hand, lower cellular calcium in chondrocytes (13) and stimulate bone accretion in patients with osteoporosis (14) and avascular necrosis (4). It is possible that PEMF's will find a variety of uses in other cases of cellular dysfunction. This study supports the hypothesis that PEMF's induce specific modifications in normal cell function. At least one effect can be directly related to transcriptional induction.

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