MIGRATION OF CELL SURFACE CONCANAVALIN A RECEPTORS IN PULSED ELECTRIC FIELDS

S. LIN-LIU AND W. R. ADEY
Research Service, Loma Linda Veterans Administration Medical Center, California 92357

M.-M. POO
Department of Physiology and Biophysics, University of California, Irvine, California 92717

ABSTRACT Concanavalin A (con A) receptors on the surface of cultured *Xenopus* myoblasts redistributed in response to monopolar, pulsed electric fields. The prefield uniform distribution of the receptors became asymmetrical, and was polarized toward the cathodal pole, in the same way as in DC fields. The extent of asymmetry depended on the duration of field exposure, pulse width (or alternatively, interpulse interval), frequency, and intensity. This relationship was most conveniently expressed by using duty cycle, a quantity determined by both pulse width and frequency. Pulses of average intensity 1.5 V/cm induced detectable asymmetry within 5 min. At the lowest average field intensity used, 0.3 V/cm, significant asymmetry was detected at 150 min. For pulses of high duty cycle (>25%), steady state was reached after 30 min exposure and the steady state asymmetry was dependent on average field intensity. For low duty cycle fields, the time required to reach steady state was prolonged (>50 min). Before reaching a steady state, effectiveness of the pulses, as compared with DC fields of equivalent intensity, was a function of duty cycle. A low duty cycle field (fixed number of pulses at low frequency or long interpulse interval) was less effective than high duty cycle fields or DC.

INTRODUCTION

The distribution of cell surface macromolecules can be modulated by various factors, such as extracellular ligands (1–3), cell-cell, or cell-substrate contacts (4–7), and extracellular electric fields (8, 9). In a number of cell types, static electric fields cause the migration and accumulation of plasma membrane macromolecules toward one side of the cell, a phenomenon termed "in situ electrophoresis" or "electromigration" (10, 11). Steady state accumulation of charged membrane components in the presence of a DC field depends upon at least three molecular processes on the cell surface: lateral electrophoresis, Brownian diffusion, and solvent drag due to the electro-osmotic flow of fluid near the cell surface (12). Although the complete picture of electro-kinetic phenomena involved in electromigration remains to be elucidated, static electric fields have provided a useful tool in perturbing membrane topography and in studying the dynamics of cell surfaces (6, 13).

Besides the use of an electric field as a tool in studying cell surfaces, there is a separate issue concerning the biological implication of electromigration, namely, is an endogenous electric field within tissue effective in modulating membrane topography? In particular, are pulsed repetitive electric fields, which are characteristic of endogenous fields associated with neural activities, capable of inducing migration of plasma membrane components? If so, what are the intensity and frequency requirements of the effective field? In the present study, we investigated the effect of pulsed fields on the surface distribution of concanavalin A (con A) receptors in cultured *Xenopus* myoblasts. Our aim was to find out whether pulsed fields induce migration of con A receptors, the time course of the response, and the effective pulse parameters. The effectiveness of pulsed fields will then be compared with that of DC fields in the same culture system. These results provide a basis for future inquiries into the effects of endogenous field on membrane topography in the nervous tissue.

MATERIALS AND METHODS

Embryonic muscle cells were obtained from *Xenopus laevis* (Nasco Corp., Fort Atkinson, WI) embryos at stage 17–19 (14). Cells were plated as monolayers on cover slips and cultured at 21°C for 40–48 h. Culture medium contained 85% Steinberg's saline (buffered with 10 mM HEPES [Sigma Chemical Co., St. Louis, MO]), 10% Leibovitz (Gibco Laboratories, Grand Island, NY) medium, and 5% fetal calf (Gibco Laboratories) serum; pH was 7.8. The electrophoresis chamber was similar to that described previously (15), except that the cells were on cover slips. Before field application, culture medium was replaced by Steinberg's saline. Electric current was applied to the chamber through a pair of agar bridges. Current and potential drop across the chamber were monitored. All experiments were performed at room temperature inside a Faraday cage. Immediately (time delay was typically 4 s) following field application, cell surface con A receptors were labeled with con A conjugated with tetramethylrhodamine isothiocyanate (R-con A) (Victor Lab Inc., Burlingame, CA). Labeling was carried out at 4°C for 8.5 min with Steinberg's saline containing 15 ug/ml R-con A and 0.1% bovine serum albumin (BSA) (Sigma Chemical Co.). Cells were then rinsed thoroughly at 4°C with Steinberg's BSA followed by Steinberg's saline alone. This was followed immediately by microfluorimetry on a Zeiss inverted

fluorescence microscope (Carl Zeiss, Inc., Thornwood, NY) fitted with a PM1 photometer. Fluorescence intensity (I) was measured on myoblasts that retained a spherical shape, with a 9.0 μm diam aperture positioned on either side of the cell periphery along the field axis. The asymmetry index (AI) of receptor distribution on spherical cells was calculated according to the method of Poo et al. (16), namely, $AI = (I_C - I_A)/(I_C + I_A)$ where I_C and I_A are corrected (background subtracted) fluorescence intensity measured at the cell periphery facing the cathodal and anodal poles, respectively. Control cultures (in the absence of field) in general showed AI below 0.05; AI above this value was taken to be indicative of detectable receptor migration.

Square pulses were used in all experiments. For each pulsed field condition, a parallel control was performed on cultures prepared at the same time from embryos of the same batch (parallel cultures). The control was always exposed to a DC field of the same average field intensity for the same duration as the pulsed field. Results obtained from these cultures were directly compared. Three independent field parameters, frequency, pulse width, and intensity, were varied. Two other related quantities, interpulse interval and duty cycle, were also used in the description of experiments. We first studied the time course of asymmetry formation in 10 and 100 Hz fields with a 50% duty cycle. The relation between interpulse interval and asymmetry formation was then explored in short term (<10 min) and long term (>20 min) exposures. For short term responses, we used either pulses of fixed width at frequencies between 10 and 100 Hz or 10 Hz fields at various pulse widths. For long term responses, fields were applied at 5 to 100 Hz with various duty cycles. The latter conditions were further extended over the frequency range 0.5 Hz to 10 KHz with a 50% duty cycle. Detailed exposure conditions are described in the results.

RESULTS AND DISCUSSION

The apparent difference between the action of a pulsed field and of a DC field is that the unidirectional electromigration of the receptors is interrupted by periods of diffusional randomization during interpulse intervals. While the theory for electromigration under the influence of a DC field has been developed (10), a theoretical analysis for pulsed fields is not yet available. The experiments described here aimed to explore empirically (*a*) the characteristics of molecular migration under uniform pulsed fields, (*b*) the minimum average field required to produce detectable asymmetry in molecular distribution, and (*c*) the effectiveness of the pulsed field as compared with a DC field of the same average field intensity, particularly when the interpulse interval occupies a large fraction of the pulse cycle.

Time Course of Asymmetry Formation

In the first set of experiments, a group of *Xenopus* myoblast cultures (prepared from the same batch of embryos) were exposed to repetitive monopolar square-pulse fields at a pulse intensity of 3 V/cm and frequencies of 10 or 100 Hz for periods ranging from 5 to 60 min. The pulse width was set at 50 and 5 ms, respectively, for 10 and 100 Hz fields, giving identical duty cycles (50%) for both conditions. The effects of electric fields on receptor distribution was examined by postfield fluorescence labeling of cell surface con A receptors with rhodamine-conjugated con A. After 5-min exposure to the pulsed field, the distribution of cell surface con A receptors on these spherical cells became asymmetric, with more con A receptors accumulated at the periphery of the cell facing the cathode. The asymmetry in con A receptor distribution was determined quantitatively by microfluorimetry (see Methods) in terms of an asymmetry index (AI), defined as the normalized difference of fluorescence intensities measured at the cathode- and anode-facing periphery of the cell. Fig. 1 shows the result from four separate groups of cultures. Asymmetry increased monotonically with the duration of the field exposure, reaching an apparent plateau in ~30 min.

The striking finding was that the action of 10 and 100 Hz fields was nearly identical, both in the time course of asymmetry formation as well as in the extent of asymmetry at the apparent steady state. It suggests that equivalent effects will be produced by field of the same average intensity (3 V/cm × 50% or 1.5 V/cm) for both the 10 and 100 Hz fields. This notion is further supported by the fact that the effect of pulsed fields was very close to that produced by a DC field of 1.5 V/cm in sets of parallel cultures (Fig. 1).

The asymmetry induced in pulsed fields was also reversible as was described for the DC exposure (16). When cells were exposed for 30 min in the above 10 and 100 Hz pulsed fields and labeled with R-con A 30 min after termination of the fields, the asymmetry index (mean ± SEM, determined from 25 cells) was found to be close to

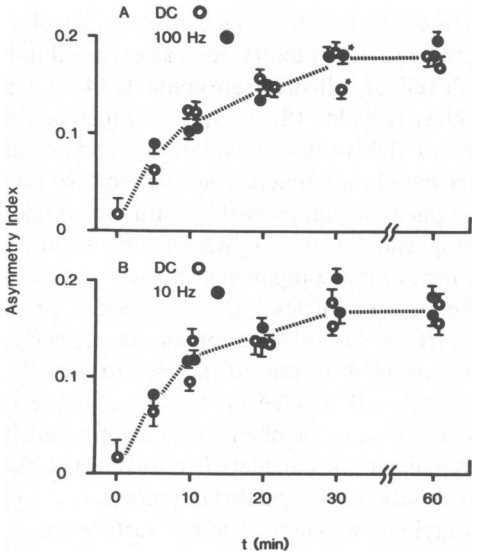

FIGURE 1 Time course for the development of asymmetry in con A receptor distribution after exposure to DC fields and 50% duty cycle pulses. Pulse intensity was always 3 V/cm and DC field intensity was 1.5 V/cm. Data show comparison between DC and (*A*) 100 Hz and (*B*) 10 Hz pulsed fields. Each data point (mean ± SEM) was determined from 22–28 cells in one culture. Each pulse-exposed culture was accompanied by a simultaneous DC exposure to a parallel culture. At each time point, these paired data were plotted with error bars pointing toward the same direction. Statistical analysis (Student's *t*-test) of the pairs showed no significant difference between the results of DC and pulsed field experiments ($P > 0.05$) except the case of marked * where $0.01 < P < 0.05$.

the baseline: 0.048 ± 0.011 and 0.040 ± 0.010 following fields of 100 and 10 Hz, respectively. The reversibility of the asymmetry after the termination of the field suggests back diffusion of the accumulated receptors (16).

For electromigration under the influence of a DC field, theoretical considerations indicated that the time required to reach steady state asymmetry (characteristic time) is directly related to the average time for the diffusion of the molecule over the dimensions of the cell on which electromigration occurs. For the cultured *Xenopus* spherical myoblasts (average diameter 35 μm), a previous study (10) has shown that both the diffusion time and $1/e$ of the characteristic time required for electromigration to reach a steady state in the DC field were ~10 min. A similar time course was also found in the present pulsed field experiments. It thus appears that, at least for the 5 and 50 ms pulses, repetitive diffusional randomization during the 5 and 50 ms interpulse intervals exerted an imperceptible influence on the time course of asymmetry development.

Frequency Dependence

A direct test of the effect of diffusional randomization during interpulse intervals is to vary the frequency of the pulsed field, while fixing all other parameters, i.e., the intensity and width of the pulse, as well as the total number of pulses delivered to the cells. Two separate experiments were carried out: first, a pulsed field of 5 ms pulse width and 6 V/cm pulse intensity was applied to a group of cultures at frequencies of 10, 20, 50, and 100 Hz for periods of 100, 50, 20, and 10 min, respectively; delivering a total of 6×10^4 pulses to each culture. In the second experiment, a similar pulse pattern was used except that the total number of pulses delivered was increased to 9×10^4. Results of these experiments are depicted in Fig. 2. We found (*a*) the asymmetry in con A receptor distribution induced by the fields of low frequency (or long IPI) was significantly lower than that induced by a high frequency field (or short IPI), even though identical amounts of total electric charge had been delivered to each culture. (*b*) the effects of 9×10^4 pulses are apparently greater than with the 6×10^4 pulses, especially for low frequency fields. Similar results were also obtained in a separate set of experiments, where a pulse intensity of 3.3 V/cm was used (data not shown).

In these experiments, the IPI of the field was inversely proportional and the average field intensity was proportional to the frequency of the field. Our finding that fields at low frequencies (10 and 20 Hz) induced lower asymmetry is consistent with the notion that long IPIs and/or low average field are associated with low asymmetry. However, one must also consider the possibility that asymmetry formation has not reached a steady state, and that the time course for attainment of steady state is frequency dependent. Note that exposures to 6×10^4 and 9×10^4 pulses at 20 Hz (average field 0.6 V/cm) involved an elapsed time of

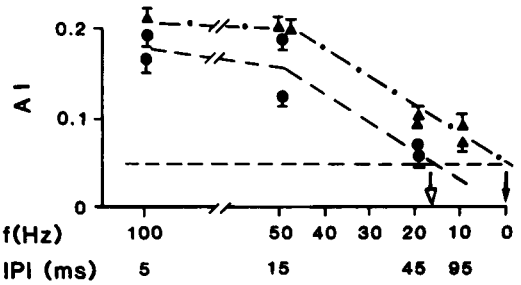

FIGURE 2 Dependence of asymmetry index on interpulse interval (IPI) or frequency of pulsed fields with fixed pulse width (5 ms), intensity (6 V/cm) and total number of pulses delivered. Pulses were delivered at various frequencies to achieve different IPIs. Although the time-averaged field intensity was lower at longer IPI, the total amount of charge passed remained the same. Within each group of experiments (6×10^4 [●] or 9×10^4 [▲] pulses), all 20 and/or 10 Hz results are statistically different from 100 and 50 Hz results ($P < 0.01$, Student's t test). Each point (mean ± SEM) was obtained from 22–28 cells in one culture. Asymmetry index of 0.05 indicates level of detectable asymmetry (straight dotted line). For 6×10^4 pulses (●), a threshold frequency between 10 and 20 Hz (↓) (IPI 50–70 ms) was obtained by extrapolation. For 9×10^4 pulses (▲), detectable asymmetry may be achieved at frequency below 10 Hz (↓).

50 and 75 min, respectively. The small but significant difference in asymmetry produced by these two treatments indeed suggests that a steady state had not been reached after 50 min exposure to a 20 Hz field. This is in contrast to the formation of asymmetry with high duty cycle fields (and high average field) shown in Fig. 1, where a clear steady state was reached within 30 min. From these experiments, we reached a tentative conclusion that for the same amount of charge delivered, high frequency pulses applied within a brief elapsed time are more effective than prolonged application of the same pulses at low frequency; and that this may be due to the fact that in the latter

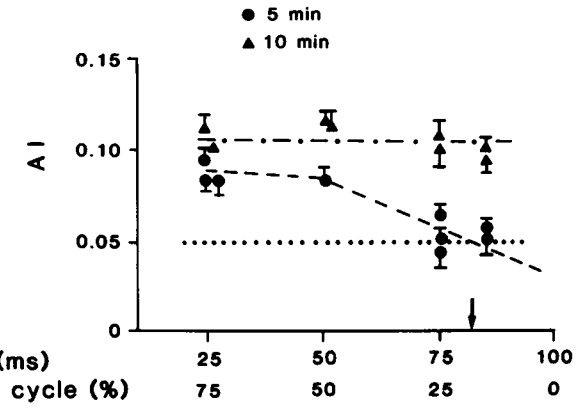

FIGURE 3 Dependence of asymmetry index on interpulse interval (IPI) of pulsed field with fixed frequency (10 Hz). Short term exposure (5 and 10 min) was examined. To keep an average field intensity of 1.5 V/cm for all IPI values used, pulse intensity was varied and was 10, 6, 3, and 2 V/cm for IPI 85, 75, 50, and 25 ms, respectively. At 5 min, all results from IPI ≥ 75 ms are statistically different from IPI = 25 ms ($P < 0.01$, Student's t test). At 10 min, the same extent of asymmetry was achieved by all field conditions. Each point (mean ± SEM) was obtained from 22–29 cells in one culture.

situation, the cells were exposed to low average field levels (and low duty cycle), or that Brownian diffusion during IPIs became significant in these low duty cycle pulses, increasing the time requirement for asymmetry formation.

Dependence on Interpulse Interval in Initial Responses

The experiments described above did not distinguish the effect of low average field and low duty cycle on the asymmetry formation. To test directly the dependence on the duty cycle, or the IPI, we carried out experiments in which the frequency was kept constant (10 Hz), and the intensity and duration of the pulse were varied to give a constant average field of 1.5 V/cm but different IPIs. The duration of field exposure was set at 5 and 10 min in two separate experiments. The result is shown in Fig. 3. For 10 min exposure, no difference in asymmetry was found for cultures exposed to different IPIs ranging from 25 to 85 ms. However, significant differences were found for cultures that were exposed for 5 min. Longer IPIs (75 and 85 ms) yielded lower asymmetry. Because all cultures experienced the same average field intensity within each cycle, the difference in asymmetry must be due to a long IPI or to the correspondingly low duty cycle. The fact that this difference was detectable at 5 min but not at 10 min suggests that the long IPI affects the initial response of asymmetry formation before the latter has reached a steady state.

In other words, the effect of IPI depends on the exposure history of the cells. This may also be true within the duration of each pulse cycle, as indicated by observations that with 5 ms pulses, an IPI of 45 ms led to a significant reduction in asymmetry (Fig. 2), whereas with 50 ms pulses, an IPI of 50 ms did not reduce the asymmetry (Fig. 3). Although average field intensity was also one determinant in these cases, it will be shown later that the relative length of pulse width and IPI (or duty cycle), rather than the absolute length of the IPI, determines the effectiveness of the pulses when compared with DC fields of the same intensity (section 6).

Steady State Asymmetry

The notion that the frequency and IPI of the pulses affects the initial response but not the steady state asymmetry was tested in two other experiments. Cultures were exposed to a long treatment (30 min) with pulsed fields of various frequencies (5 to 100 Hz) and of either 25 or 75% duty cycle. The average field intensity was kept constant at 1.5 V/cm by varying the width and intensity of the pulse for each condition. The result is shown in Fig. 4. In general all conditions resulted in asymmetry which was not significantly different from the effect of a 1.5 V/cm DC field.

Together with the result shown in Fig. 1, where a 50% duty cycle field was used, we conclude that for fields with a

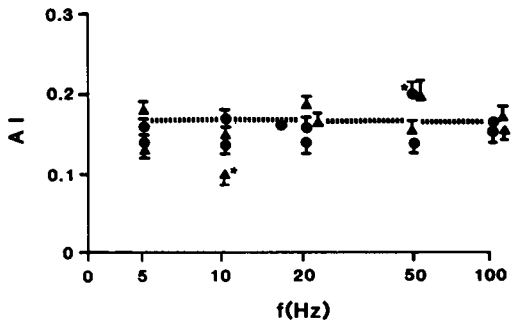

FIGURE 4 Steady state asymmetry in pulsed fields of various frequencies. Cultures were exposed for 30 min in pulsed fields of average intensity 1.5 V/cm. Pulse intensity was 6 and 2 V/cm for duty cycle 25% (▲) and 75% (●) respectively. The majority of data points scattered between *AI* of 0.14 and 0.20. For each day of experiment (typically three pulsed field exposures at different frequencies), one culture was also exposed to DC field of intensity 1.5 V/cm for 30 min. Asymmetry index from these cultures also fell in the same range. Individual result from DC exposure is not shown explicitly but the overall mean (0.168 ± 0.013, 7 cultures) is represented by the horizontal dotted line. Statistical analysis (Student's *t* test) of data from DC and pulsed field exposure obtained at the same day show no significant difference at 95% confidence level ($P > 0.05$) except for cases marked with * where $0.01 < P < 0.05$. Note that comparison was not made between ● and ▲. Each point (mean ± SEM) was obtained from 22–28 cells in one culture. (log *f* scale on abscissa).

duty cycle above 25%, the time required to reach a steady state asymmetry was <30 min. For shorter duty cycles, as used in studies in the Frequency Dependence section, namely 5 and 10%, the time course was apparently prolonged significantly, since there was an apparent increase in asymmetry when the field application was prolonged from 50 to 75 min.

Threshold Fields

An immediate question of physiological interest is the minimal field required to produce asymmetry in receptor distribution. If the field is allowed to act for a relatively long period, 10 Hz pulses of intensity 6 V/cm and width 5 ms can produce detectable asymmetry ($AI \geq 0.05$) within 150 min (Fig. 2, 9×10^4 pulses). This corresponds to a field of 5% duty cycle and an average intensity of 0.3 V/cm within each cycle. A higher frequency field was required for rapid induction of asymmetry. Thus, 100 Hz pulses of width 5 ms (50% duty cycle) and lower pulsed intensity (3 V/cm, average 1.5 V/cm) produced significant asymmetry within 5 min (Fig. 1).

Neural action potentials and synaptic potentials typically generate pulsed fields with widths in the range 1–5 ms, and at frequencies below 200 Hz. Their effectiveness in modulation of the topography of the membrane receptors depends crucially on two factors that are difficult to assess at present; the intensity of the perimembrane field within the narrow intercellular spaces or its components within the cytoplasm, and the extent of the spread of the field along the plasma membrane (18). The former relates directly to the intensity of the average field, and the latter

determines the time course of asymmetry development. A semi-analytical treatment of these problems will be presented elsewhere (Young, S. H., and M.-M. Poo, in preparation). It is worth noting, however, that a strong effect is most likely to occur where higher frequency pulses are produced in regions of the nervous system and where pulsed fields decrement rapidly over a short distance, a situation exemplified by the synaptic activity at the dendritic spine of the mammalian CNS (17).

Comparison with DC Fields: Dependence on Duty Cycle

In most of the above experiments, parallel cultures were also exposed to DC fields of the same average field intensity. A direct comparison of the effectiveness of pulsed fields with DC fields can be made. Fig. 5 A shows the result of such a comparison for pulsed field data depicted in Fig. 3. Asymmetry indices for cultures exposed to pulsed fields of various duty cycles for 5 or 10 min were divided by those of the cultures treated with a DC field of 1.5 V/cm for 5 or 10 min, respectively. A ratio of 1 indicates effectiveness the same as a DC field. Interestingly, significant differences were found for 5 min exposure, but not for 10 min exposure. Lower duty cycle fields produce less asymmetry than DC field, while the converse may be true for high duty cycle fields. A clear understanding of this behavior requires further theoretical analysis of the pre–steady state distribution of receptors during pulsed field exposure.

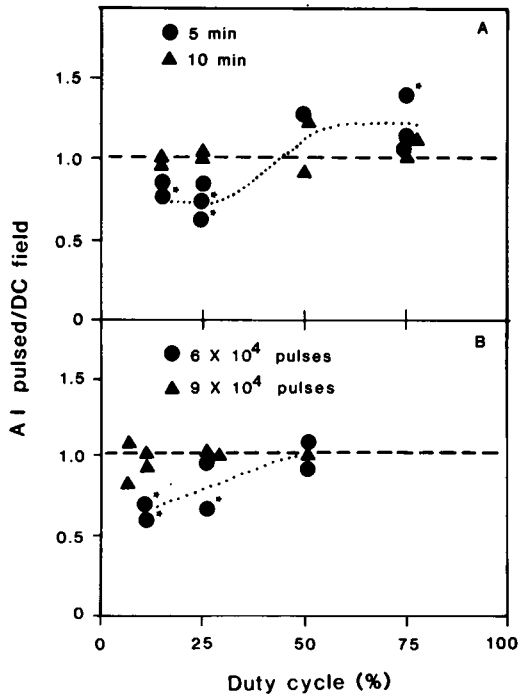

FIGURE 5 (*A*) Effectiveness of 10 Hz pulsed fields with various duty cycles. Each point represents the ratio of two mean *AI* (22–28 cells each) determined from two parallel cultures, one exposed to pulsed and the other to DC field. The pulsed field conditions were the same as those of Fig. 3. DC field intensity was 1.5 V/cm. (*B*) Effectiveness of 5 ms pulses delivered as fields of various duty cycles. Each point represent the ratio obtained similar to those of *A*. Pulsed field conditions were the same as those in Fig. 2. DC field intensity corresponded to the average field intensity of the pulsed field and was 0.3, 0.6, 1.5, and 3 V/cm for duty cycle 5, 10, 25, and 50%. In both *A* and *B*, horizontal dotted line indicates results from DC exposure in the corresponding conditions. Student's *t* test on paired results (before taking ratio) from DC and pulsed fields showed statistically significant differences at 95% confidence level ($P < 0.05$) for points marked with * to their *upper right*.

A similar situation was found for the pulsed field study described in section 2 (Fig. 2). Asymmetry resulting from exposure to the 20 Hz, 5 ms pulses for 50 min (Fig. 5 B, total 6×10^4 pulses, duty cycle 10%) is less effective than that produced by DC fields of the same average field intensity, while longer exposures (75 min, 9×10^4 pulses, duty cycle 10%) were as effective as exposure to equivalent DC fields. Because, for this low duty cycle (10%) field, the steady state apparently has not been reached by 50 min (see discussion in section 2), this result is consistent with the notion that the effectiveness of the pulsed field differs from that of the equivalent DC field only during pre–steady state conditions.

Common features of Fig. 5, *A* and *B*, not only showed that the effectiveness of pulsed fields (before steady state) is dependent on duty cycle, but also suggested a critical value of the duty cycle at around 25%. For duty cycles below this value, pulses were less effective than DC fields or pulses of duty cycle above 50%. A particularly interesting indication of these results is that when the same amount of charge is contained in each pulse, wider pulses (e.g., 75% duty cycle) are more effective than narrow pulses (e.g., 25% duty cycle). An hypothetical field condition can be made in which an asymmetric AC field is composed of pulses of opposite polarities, occupying 10 and 90% of the cycle time and of intensity ratio of 9:1. Overall current generated during each cycle is zero. With prolonged exposure to such a field, no induced asymmetry would be expected, since the average field intensity is zero. However, with brief exposure to trains of such pulses we would expect that a small asymmetry could be induced if effects of the pulses of the two opposite polarities are additive. Our efforts in direct experimental detection of such an effect were not successful because at the present pulse intensity used, the extent of asymmetry, if it exists at all, was very close to noise level. Nevertheless, it is worth noting that the local extracellular electric fields generated by a neural action potential is likely to resemble this kind of AC field (e.g., 100 Hz activity with 1 ms depolarization and longer hyperpolarization >5 ms).

It should also be noted that because diffusional randomization of con A receptors is highly temperature dependent (10), we may expect the critical duty cycle to also show temperature dependence. At high temperature, fast diffusion of the receptors probably lead to a higher critical value, whereas the reverse may be expected at low temperature.

To further test whether a pulsed field is as effective as an equivalent DC field in near steady-state conditions, we compared the asymmetry produced by 20 min exposure with a 50% duty cycle field over a wide frequency range (0.5 Hz to 10 KHz) with effects of DC fields, for two different average field intensities (2 and 3.8 V/cm). The result is shown in Fig. 6. No statistically significant difference was observed, ($P > 0.05$) between DC and pulsed field except in one case where $0.01 < P < 0.05$ was found.

CONCLUSIONS

The present experiment indicates that the effectiveness of pulsed fields in producing net electromigration of receptors on cell surfaces can be considered in two aspects: the first concerns the characteristics of the applied field, namely its time-averaged field intensity and duty cycle of the pulses; the second concerns the process of migration per se, namely, whether or not the migration has reached steady state. In the steady state condition, pulsed fields of different patterns but the same average field intensity produce the same effect, which is also identical to that produced by equivalent DC fields. In the pre–steady state, the lower duty cycle field is less effective than the higher duty cycle field of the same average field intensity, as well as the equivalent DC field. Frequency dependence in pre–steady state is implied by these factors. Because the time course of the approach to steady state is apparently prolonged for low duty cycle, low frequency fields, brief pulses (e.g. 5 ms) delivered at high frequency (e.g. 100 Hz), are more effective than at low frequency (e.g., 10 Hz), even though the same total number of pulses is delivered.

The present study was carried out on a model spherical cell type, using a uniform electric field. Because the time course of approach to steady state depends directly on the spatial dimension of the membrane within which the receptor migration takes place, the results obtained in this system cannot be applied quantitatively in other systems where a pulsed field occurs in a localized, nonuniform fashion. Recent computer modeling studies on migration in a localized, decremental pulsed field (S. H. Young, personal communication) suggest that the behavior of migrating receptors is qualitatively similar to the situation for the uniform field described here.

Action potentials and synaptic potentials in the nervous system are characterized by brief pulses on the order of milleseconds and at frequencies up to 200 Hz. The effect of such fields in causing migration of membrane molecules, if it exists at all, is likely to be frequency dependent. Besides the apparent difference in average field intensities at different frequencies for these brief pulses, there is a further drop of effectiveness for low-frequency (e.g., 10 Hz) fields, due to prolonged pre–steady state.

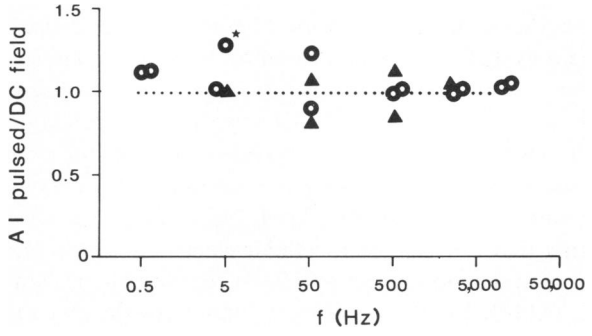

FIGURE 6 Effectiveness of 50% duty cycle pulses over an extended frequency range (0.5 Hz to 10 kHz). Each point represents the ratio obtained similar to those of Fig. 5. Horizontal dotted line indicates results from DC field exposure. DC field intensity was 1 and 1.9 V/cm for pulses 2 (▲) and 3.8 (○) V/cm, respectively. Exposure duration was 20 min. Comparison between DC and pulsed field exposure showed no statistically significant difference at 95% confidence level ($P > 0.05$) except for the case marked with * to its upper right, where $0.01 < P < 0.05$.

The authors wish to thank Mr. E. M. Helm for valuable technical assistance. This work was supported by Department of Energy contract DEAI0179ET29078, Food and Drug Administration National Center for Devices and Radiological Health contract 5R01FD00963-06 and Southern California Edison Company contract C0033902 to W. R. Adey and National Institutes of Health grant NS-17558 to M.-M. Poo.

Received for publication 6 July 1983.

REFERENCES

1. de Petris, S., M. C. Raff, and L. Mallucci. 1973. Ligand-induced redistribution of concanavalin A receptors on normal, trypsinized, and transformed fibroblast. *Nat. New Biol.* 244:275–278.
2. Haigler, H. T., J. A. McKanna, and S. Cohen. 1979. Direct visualization of the binding and internalization of a ferritin conjugate of epidermal growth factor in human carcinoma cells A-431. *J. Cell. Biol.* 81:382–395.
3. Christian, C. N., M. P. Daniels, H. Sugiyama, Z. Vogel, L. Jacques, and P. G. Nelson. 1978. A factor from neurons increases the number of acetylcholine receptor aggregates on cultured muscle cells. *Proc Natl. Acad. Sci. USA.* 75:4011–4015.
4. Anderson, M. J., and M. W. Cohen. 1978. Nerve-induced and spontaneous redistribution of acetylcholine receptors on cultured muscle cell. *J. Physiol. (Lond.).* 268:757–773.
5. Johnson, R., M. Hammer, J. Sheridan, and J. P. Revel. 1974. Gap junction formation between reaggregated Novikoff hepatoma cells. *Proc. Natl. Acad. Sci. USA.* 71:4536–4540.
6. Chow, I., and M.-M. Poo. 1982. Redistribution of cell surface receptors induced by cell-cell contact. *J. Cell Biol.* 95:510–518.
7. Moody-Corbett, F., and M. W. Cohen. 1982. Increased adhesiveness at sites of high acetylcholine receptor density on embryonic amphibian muscle cells cultured without nerve. *J. Embryol. Exp. Morph.* 72:53–69.
8. Poo, M.-M., and Robinson. 1977. Electrophoresis of concanavalin A receptors along embryonic muscle cell membrane. *Nature (Lond.).* 265:602–605.
9. Orida, N., and M.-M. Poo. 1978. Electrophoretic movement and localization of acetylcholine receptors in the embryonic muscle cell membrane. *Nature (Lond.).* 275:31–35.
10. Poo, M.-M. 1981. In situ electrophoresis of membrane components. *Annu. Rev. Biophys. Bioeng.* 10:245–276.
11. Fraser, S. E., and M.-M. Poo. 1982. Development, maintenance, and

modulation of patterned membrane topography: models based on acetylcholine receptors. *Curr. Top. Dev. Biol.* 17:77–100.

12. McLaughlin, S., and M.-M. Poo. 1981. The role of electro-osmosis in the electricfield-induced movement of charged macromolecules on the surfaces of cells. *Biophys. J.* 34:85–93.
13. Young, S. H., and M.-M Poo. 1983. Topographic rearrangement of acetylcholine receptors alters channel kinetics. *Nature (Lond.).* 304:161–163.
14. Nieuwkoop, P. D., and J. Faber. 1967. Normal table of *Xenopus laevis*. Elsevier-North Holland Publishing Co., Amsterdam. Second ed.
15. Poo, M.-M., W.-J. H. Poo, and J. W. Lam. 1978. Lateral electrophoresis and diffusion of concanavalin A receptors in the membrane of embryonic muscle cell. *J. Cell Biol.* 76:483–501.
16. Poo, M.-M. J. W. Lam, N. Orida, and A. W. Chao. 1979. Electrophoresis and diffusion in the plane of the cell membrane. *Biophys. J.* 26:1–22.
17. Jack, J., D. Noble, and R. Tsien. 1975. Electric Current Flow in Excitable Cells. Oxford University Press, Oxford, 218–223.
18. Elul, R. 1967. Fixed charge in the cell membrane. *J. Physiol. (Lond.).* 189:351–365.