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Biophoton emissions from cell cultures: biochemical evidence for the plasma membrane as the primary source

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Abstract. Photon emissions were measured at ambient temperature (21°C) in complete darkness once *per* min from cultures of 10^6 cells during the 12 h following removal from 37°C. The energy of emission was about 10^{-20} J/s/cell. Of 8 different cell lines, B16-BL6 (mouse melanoma cells) demonstrated the most conspicuous emission profile. Acridine orange and ethidium bromide indicated the membranes were intact with no indication of (trypan blue) cell necrosis. Treatments with EGF and ionomycin produced rapid early (first 3 h) increases in energy emission while glutamine-free, sodium azide and wortmanin-treated cells showed a general diminishment 3 to 9 h later. The results suggested the most probable origin of the photon emission was the plasma cell membrane. Measures from cells synchronized at the M- and S-phase supported this inference.

Key words: Biophoton emission — B16 (melanoma) cells — Membrane — EGF — Cell culture

Abbreviations: BIS, bisindolymaleimide; CsA, cyclosporin A; EGF, epidermal growth factor; PMT, photomultiplier tube.

Introduction

Spontaneous photon or "biophoton" emission has been described as ultraweak packets of electromagnetic energy (Popp 1979). Many recent publications have reported the role these biophotons play in biological systems (Belousov et al. 2000; Bajpai 2003), looking at emission intensities from cultures of plant and animal tissues (Kataoka et al. 2001; Belousov 2003; Winkler et al. 2009). Cultures of living cells emit biophotons whose intensities have been measured to be within the order of 10^{-12} W/m^2 (Isojima et al. 1995) or 10^6 to 10^7 photons/m^2/s (Popp et al. 1988; Tilbury and Quickenden 1988). By use of photomultiplier tubes (PMT) emissions have been detected from all organs measured and are coupled to function (Inaba 1988; Chang 2008). For example, the magnitudes of emissions are related to the brain's physiological state and are even coupled in hippocampal slices to the proportion of theta (4 Hz to 7 Hz) activity, its intrinsic electrophysiological variation (Kobayashi et al. 1999).

As imagined by quantum physicists such as Schroedinger (1944) and Bohr (1958), biophoton emissions have been considered an essential electromagnetic basis to life. Their role in intracellular and extracellular exchange of information as conceived by Popp (1979), particularly in light of the observations photons may not be massless (Tu et al. 2005), encourage additional explanations for the fundamental physicochemical properties of cellular molecular processes. Biophotons as neural communication signals (Sun et al. 2010) could significantly alter interpretation of brain function. Estimates (Bókkon et al. 2010) indicate that 10^8 biophotons/s can be produced inside retinotopic visual neurons. In fact Bókkon (2005) has hypothesized that during dreams the visual images may actually be fields of photon emissions.

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A primary source of biophotons has been attributed to free radical reactions involving reactive oxygen and nitrogen species (ROS and RNS) produced primarily by the mitochondrial oxidative metabolism and lipid peroxidation (Scott et al. 1991). While monitoring B16-BL6 (B16) cells (mouse melanoma cells) at room temperature (21°C) after removal from 37°C we measured energy emissions that were within the range expected from changes in membrane potentials (Persinger 2010). As indicted by acridine orange and ethidium bromide (by which membrane permeability is often inferred), we found the cell membranes still maintained their properties 12 h after removal from their incubated environment and placement within the experimental room (21°C, darkness). In fact 24 h later only a very small proportion of the population were dead as defined by trypan blue.

During this 12 h period we observed reliable and robust increases in photon emissions. This elevation continued until the vitality diminished and was equivalent to about 10^{-20} J *per* cell/s. This is the amount of energy from a potential difference of 0.07 V (of a membrane) on a unit charge (1.6×10^{-19} As) and has been considered an essential quantal unit in the physical chemistry of cell function (Persinger 2010). We present here evidence that the source of this emission may be from the plasma cell membrane.

Materials and Methods

Cells

The following cells were studied: B16-BL6 cell line, an aggressive murine melanoma (American Type Culture Collection, USA), MDA-MB-231 (MDA; breast cancer), HBL100 (HBL; normal breast), HEK-293 (HEK; Human embryonic kidney), HSG (Human salivary gland), SK-Mel-23 (SK-Mel), THP-1 (THP), HELA, and U937 cells.

Chemicals and reagents

The inhibitors wortmanin, bisindolymaleimide (BIS), PD98059, forskolin and SQ22536 were obtained from EMD Calbiochem while cyclosporin A (CsA), verapamil, and all other compounds were obtained from Sigma.

Procedure

Over a one-year period all cells were transported in the same manner from the incubator at the hospital laboratory to the biophysics area in another building. Cells were maintained in 150 × 20 mm tissue culture plates (Sarstedt, Laval, USA) in Dulbecco's Modified Essential Medium (DMEM, Hyclone, Logan, USA) supplemented with 10% fetal bovine serum, 100 ug/m streptomycin, and 100 U/ml penicillin (Invitrogen, Burlington, USA). Cells were incubated at 37°C in 5% CO_2 and subcultured 1 : 5 about every 3 to 4 days. The cell monolayers were washed with PBS (pH 7.4) and harvested by incubation in 0.25% trypsin-EDTA and then collected by centrifugation at 1000 × *g* for 10 min. Cells were seeded onto 60 × 15 mm tissue plate cultures and grown to maximum confluence (approximately 1 million cells). All treatments (concentrations noted below) were added to the tissue culture plates 1 h prior to the placement within the experimental room.

Within 30 to 60 min after removal from the incubator the single plate of 10^6 cells was placed directly over the aperture (8 cm^2) of the sensor of a PMT. The sensor was located in a box whose inner surface has been painted black which was covered by 10 layers (8 cm) of thick cotton cloth. Unless stated otherwise, the energy emission from the PMT was measured once in every min and recorded by a laptop computer for 12 h. The sampling time of 1 *per* min allowed sufficient data (720 points) to discern any potential patterns in intrinsic fluctuations within the population yet not overload recording instruments.

For the first part of the experiment energy measurements were completed for 8 different cells lines as well as the B16s in order to discern differences in output and intrinsic temporal variations while responding to the removal from 37°C. The lines were HEK, HSG, SK-Mel, THP, HELA, U937, MDA and HBL. The minute-to-minute measurements were also spectral analyzed by fast Fourier transformation to detect potential "signatures" or different power distributions for the frequency of photon emissions between cell lines. Because the B16s showed the most robust and highest amplitude spectral variations as well as energy output we systematically assessed these cells.

In the second part of the experiment various treatments were administered to only the B16 cell cultures. A total of three replicates, at different times over the year, were completed for each treatment. We appreciated the multiple molecular pathways potentially involved with our observations when designing the experiments. All concentrations were selected from past practice or according to the typical values published in the scientific literature. To emphasize general metabolism cells were placed in environments that were glutamine-free (Glu-free) or containing sodium azide (20 uM); the latter mitochondrial inhibitor produces metabolic death within about 4 h.

For more membrane-related effects epithelial growth factor (EGF; 20 uM), which activates MAP-kinase through the EGF receptor on the membrane and ionomycin (20 μM) which produces punctuate loss of the selective permeability of the membrane, were selected. In addition, wortmanin (5 μM) was used to inhibit PI3 kinase, PD-98059 (10 μM) to inhibit MEK in MAP-kinase, BIS (5 μM) for PKC inhibition and CsA (20 μM) to affect the calcineurin pathway. They were compared with the more specific cyclic AMP modulators

SQ22536 (10 μM) and forskolin (20 μM). To assess potential Ca^{2+} participation, NiCl (100 μM) and verapamil (100 μM) which inhibits L-type channels and fendiline (50 μM) that has a similar effect on L- or T-type channels were tested.

The results of these experiments required further assessment of photon emission during the cell cycle. In part 3, B16-BL6 cells were synchronized at M-phase using a Thymidine-Nocodazole method. Cells were grown to approximately 40% confluence. Thymidine (2 mM) was added to the media and the cells were incubated for 24 h. The media was then removed and cells were washed 3 times in PBS. Fresh media (without thymidine) was added for 3 h to release the cells. Nocodazole (100 ng/ml) was added to the medium and cells were incubated for 12 h. Cells were washed with PBS and fresh media was added to release the cells. Samples were collected at various one hour time points in order to track progression through the cell cycle. A synchronized sample was also added to the PMT and measurements were taken.

For flow cytometry analysis cells were collected at times 0, 1, 2, 4, 8, 12, 16, 20 and 24 h following release of the cell cycle blocks. Cells were washed with PBS and harvested by incubation in 0.25% trypsin-EDTA and then collected by centrifugation at 1000 × *g* for 10 min. The cell pellets were fixed in 70% ethanol at –20°C overnight. After being washed twice with PBS the cells were stained with propidium iodide (PI) solution (RNAase 100 pg/ml, PI 50 pg/ml diluted in PBS, pH 7.4).

To verify cell proliferation, the Trypan blue exclusion assay was used to determine if cells grown in the absence of the optimal incubator conditions (37°C and 5%) had significantly higher levels of dead cells when compared to cells remaining in the incubator. Cells were grown onto replicate plates and collected at various sequential one hour time points. At each time point a replicate plate of cells was harvested by incubation in 0.25% trypsin-EDTA and then collected by centrifugation at 2000 rpm for 10 min. The pellets were resuspended in PBS (pH 7.4) treated with 15 μl of 1% Trypan blue and counted using a haemacytometer. Eight separate counts were performed for each sample.

To assess the presence of apoptosis and membrane integrity, acridine orange staining was used to detect morphological changes in the membrane and the presence of apoptotic cells (Pearce et al. 2001). Cells were plated on glass cover slips overnight at 37°C to allow the cells to adhere. One set of cells was placed outside of the incubator to replicate conditions similar to that of cells placed on the PMT and the other set was left in the incubator. The cells were collected at hourly time points washed in PBS and stained with 50 μl of staining solution (5 μg/ml acridine orange (Sigma) and 5 μg/ml ethidium bromide (Sigma) in PBS) for 10 min. The cover slips were mounted on a glass slide and sealed with clear nail polish. Images of the stained cells were taken at various magnifications using an Axiovert fluorescence microscope.

Measurements

A Model 15 Photometer with a meter containing a unit scale between 1 to 100 from SRI Instruments (Pacific Photometric Instruments) with a PMT housing (BCA IP21) for a RCA electron tube (no filters) was calibrated directly and indirectly. At higher intensities (>1 lux) values were measured by digital luxmeters while lower intensities were determined by responses to a 700 nm LED at 10 mA (5 millicandella; 2 millilumens/45 degree) at various distances. The lux values were transformed to Watts/m^2. Calibration indicated that a change of 1 unit (along 1 to 100 unit scale) of the PM was equivalent to 5×10^{-11} W/m^2 or about 10^{-14} J/s *per* group of cells within the area of their containers.

The output was transformed to mVm (millivolt meter) and sent to an IBM ThinkPad laptop (Windows 95) set to obtain 1 sample every 60 s unless specified otherwise. From this sample a median and mode were computed for each 12 h run and then each measure was subtracted from that reference. Measures for each of the four 3-h blocks were also computed for the 12 h. In each block the sum of all units above zero were computed and converted into energy equivalents.

Data analyses

Spectral analyses for the periodicities in photon emission for each of the 8 cell types were completed by the Plotter Program. One way analyses of variance were completed between the 15 treatments for the measures of photon emission *per* block and for all three blocks. The post hoc test was Tukey set at $p < 0.05$. All analyses involved SPSS software on a Vax Computer.

Results

General photon emission from B16 cells

Figure 1 shows a typical display of the raw measurements from the PMT over time (3 samples *per* s) from 10^6 B16 cells for the first approximately 3 h after removal from incubating temperatures. One unit is equivalent to 5×10^{-11} W/m^2. For comparison the emission measured for medium only is also shown.

The downward drift during the first approximately 30 min is typical of equilibration with the ambient temperature (21°C) while the sudden transient increases in photon emission around 2000, 3000, 4000 s and the wider and more elevated increases after 5000 s (about 1.5 h) were highly reliable patterns that were the subject of the study.

Spectral analyses of different cell lines

Comparisons of the spectral analyses of the variations in energy emissions from 10^6 cells from seven different cell

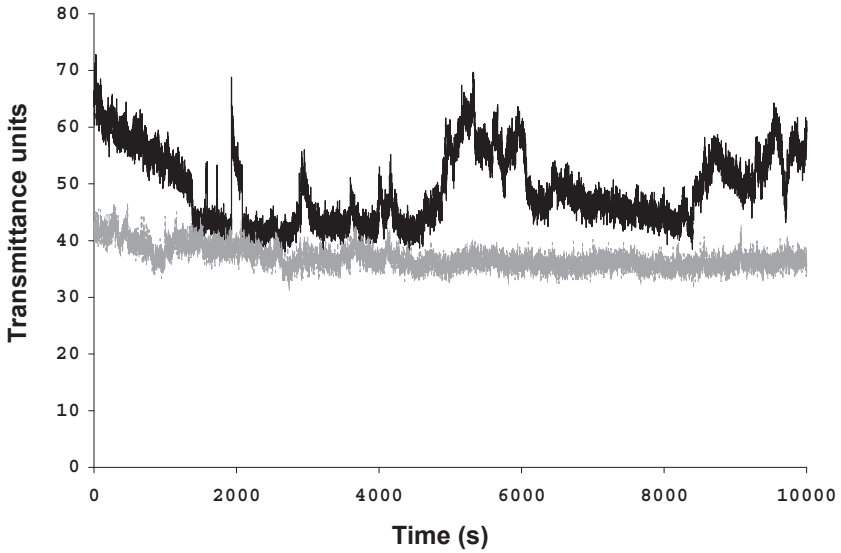

Figure 1. Direct (raw) transmittance (each unit = 5×10^{-11} W/m^2) measured by the Photomultiplier Tube (PMT) over time after removal from incubation for a plate of 10^6 B16 cells (top variable photon emissions) compared to a serum only (no cells) plate at room temperature. Sampling rate was 3/s.

lines are shown in Figure 2. Most of the variation in photon emissions from these cells occurred between the frequency equivalence approaching the duration of measurement and 1 mHz (about 16.6 min). From the perspective of temporal patterns, different cell lines displayed relatively consistent and visually discriminably different profiles.

Figure 3 shows the spectral power for the B16 cells and HEK cells that were similar to cells shown in Figure 2, for comparison. The elevated power values for the B16 cells were due primarily to the narrower range in peak power distribution across the mHz range compared to all other cell lines. In addition the inflection for diminished

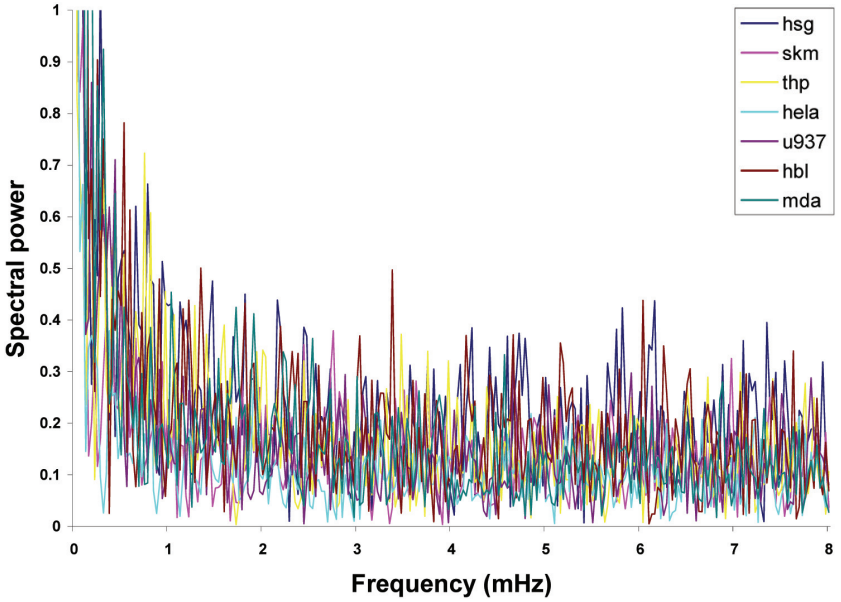

Figure 2. Spectral analyses of photon emission energies sampled once *per* min for seven different untreated cell types. The inflection approx. 1 mHz (or 15 min) indicates that the greater power emissions occurred in peaks that were between the duration of the measurements (about 12 h and about 3 h).

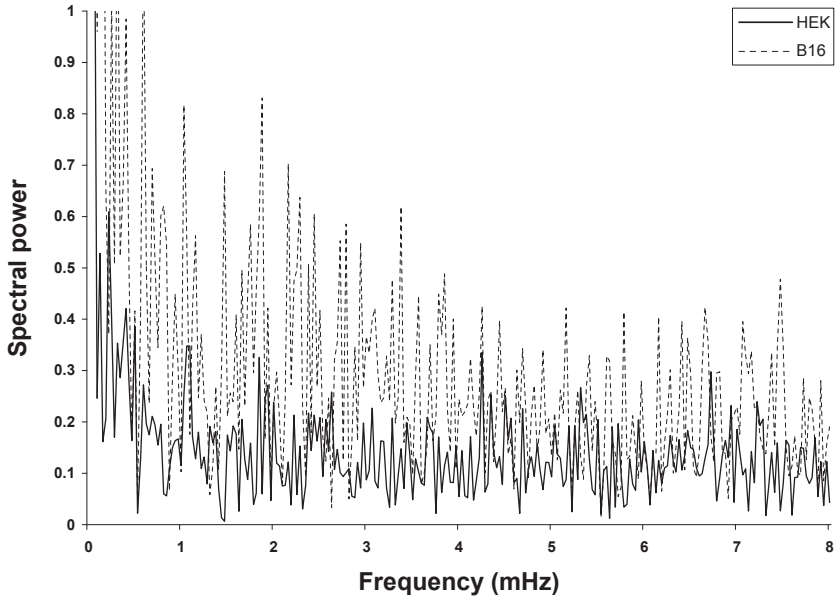

Figure 3. Spectral analyses of photon emission energies sampled once *per* min for an eighth cell type (with values similar to the seven types in Figure 2) and the B16 cells. Note the B16 cells displayed about twice the power in discrete peaks within an asymptote and periods about 4 times faster than other cells.

power for the B16 cells occurred around 5 mHz (about 3.3 min).

Treatment effects

The means and standard deviations for the energy levels emitted by B16 cells exposed to 15 different treatments (total of 45 runs on separate days) during each of the successive 3 h increments for 12 h for the triplicates for each treatment are shown in Table 1. The measures refer to the sum of energy for all cells during the three hour interval. For the first block of the untreated B16 cells the value of 7.75×10^{-10} J divided by 1.08×10^4 s (3 h) and this quantity divided by 10^6 cells is in the order of 10^{-20} J/s *per* cell which is congruent with calculated estimations (Persinger 2010). The order of magnitude was 10^5 less than what might be expected from metabolic activity of about a pJ/s *per* cell. With approximately half the number of cells (indicated by "half" on tables) the output was about half as much and not statistically significant considering the standard deviations.

The most conspicuous effect was the comparable (within a factor of about 6) energy emission from all cell cultures regardless of treatment over the 12 h of measurement. As indicated in Table 1 the total energy associated with the untreated (straight) cells and those pretreated with EGF were conspicuously higher than those treated with NaN_3 or those in Glu-free medium. The means of the two pairs of measurements were separated by about 11 standard deviations $[(X_a - X_b)/SD_{ave}]$.

The post hoc results from the analysis of variance for comparisons of all treatment groups are shown in Table 2. Asterisks indicate significant differences ($p < 0.05$) between this group and the orthogonal group. Clearly the Glu-free and sodium azide group showed overall less energy emission than the reference (straight), EGF, ionomycin and verapamil treated groups.

There were also quantitative and qualitatively obvious differences for when, over the 12 h period, the changes in energy emission occurred. The clearest effect occurred with the EGF treatment. As seen in Figure 4 most of the energy emission occurred during the first 3 h and was then followed by a rapid diminishment. However, the total energy emitted during the 12 h was almost identical to the untreated (straight) cells. In other words, about the 4-fold amount of energy was emitted during the first 3 h.

Statistical analyses confirmed this qualitative effect. One-way analysis of variance (ANOVA) for all 15 treatments demonstrated statistically significant differences($F(14.43) = 3.80$, $p = 0.001$) for the first block. Post hoc tests (Tukey's $p < 0.05$) indicated that there was more photon energy emission from the EGF-treated cells than in the cells from the Glu-free, sodium azide, NiCl, BIS, SQ, PD, or forskolin treatments that did not differ significantly from each other. Emissions from cells from the other treatments were intermediate values. The EGF effect accounted for about 66% (η^2) of the variance in our measures. The absence of significant differences in the tests of homogeneity of variance between groups eliminated the contribution from outliers and re-af-

Table 1. Means and standard deviations for the energy levels emitted by B16 cells exposed to 15 different treatments

Condition	Block 1		Block 2		Block 3		Block 4		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Straight	7.75	3.62	9.62	1.89	6.55	2.88	2.45	1.73	26.37	1.38
EGF	11.32	4.66	7.48	5.72	3.64	3.90	3.91	3.45	26.36	3.92
Ionomycin	8.45	4.49	5.88	4.78	3.37	2.47	3.03	0.12	20.74	10.41
Verapamil	5.01	3.02	3.66	5.35	4.17	2.26	2.73	1.56	15.57	3.22
CsA	3.79	1.47	2.87	0.63	2.10	0.78	4.26	1.34	13.02	1.71
Forskolin	1.72	1.53	3.03	1.57	2.15	1.49	4.61	2.58	11.52	1.89
Half	3.25	1.80	4.17	1.06	1.79	1.72	1.68	1.61	10.90	1.98
Fendiline	4.12	4.96	2.35	1.82	1.74	1.20	0.99	0.29	9.21	2.64
SQ 22536	1.15	0.09	2.82	0.48	2.31	1.49	1.04	1.06	7.33	2.06
Nickel chloride	2.13	1.06	1.93	0.67	1.66	1.62	1.33	0.84	7.05	3.47
Wortmanin	3.31	2.24	1.30	1.11	0.51	0.54	0.77	0.79	5.89	0.49
PD	1.35	1.17	1.53	0.60	1.45	0.02	1.36	1.11	5.69	0.63
BIS	0.44	0.08	2.49	2.07	2.27	1.56	0.46	0.66	5.66	3.62
Glu-free	1.94	2.26	0.68	0.03	0.92	0.80	0.78	0.39	4.32	2.31
Sodium azide	1.41	0.73	0.92	0.55	0.75	0.14	1.22	0.69	4.30	1.02

Data is presented in blocks each with a 3 h increment for 12 h for the triplicates for each treatment. The measures refer to the sum of energy for all cells during the three hour interval. Energy value for each block was 10^{-10} J. For more details, see in the text.

firmed the consistency of the measurements between and within the triplicates.

For the second time block of 3 h one-way ANOVA showed significant ($F(14.43) = 2.66$, $p < 0.01$; $\eta^2 = 50\%$ of variance explained) treatment differences. Post hoc analysis indicated that during this period the Glu-free, sodium azide and wortmanin treated cells displayed less mean energy emission than the reference (straight) cells. For this block there was also greater heterogeneity of variance between treatments (Bartlett's Box = 3.18, $p < 0.01$) due to the greater within treatment variability for the EGF, ionomycin, and verapamil treated cells compared to the Glu-free, sodium azide, NiCl, SQ, CsA and PD treated cells.

There was still significantly lower emission from cells treated with no glutamine, sodium azide, or wortmainin during the third block (6 to 9 h) compared to the untreated cells ($F = 2.07$, $p < 0.05$). During the fourth block the standard deviations of the measurements for most groups approached the value for the means. The primary source of the significant group differences was due to the greater emission for the CsA group compared to the reference.

Table 2. Post hoc results from the analysis of variance for comparisons between all treatment groups

	Straight	EGF	Ionomycin	Verapamil
Straight				
EGF				
Ionomycin				
Verapamil				
CsA	*			
Forskolin	*			
Half	*			
Fendiline	*	*	*	
SQ 22536	*	*	*	
Nickel chloride	*	*	*	
Wortmanin	*	*	*	
PD	*	*	*	
BIS	*	*	*	
Glu-free	*	*	*	*
Sodium azide	*	*	*	*

Asterisks indicate significant differences ($p < 0.05$) between horizontal group (rows: Straight, EGF, Ionomycin, Verapamil) and the orthogonal group.

Cell Cycle

The mean energy increases *per* min for the G1, G2, S, and M phases were 1.0, 1.9, 2.5 and 4.3, and 1.7, 2.3, 4.3, and 4.5, respectively, 10^{-12} J/min for the double thymidine (S phase) and thymidine-nocodazole (M phase). There were between 120 and 360 samples (1/min) *per* phase and with typical standard deviations of 0.3 to 0.4, the approximately doubling of the energy output during the S and M phases were statistically significant.

Discussion

Spontaneous emissions of "biophotons" from cell cultures (Popp 1979), tissue preparations (Isojima et al. 1995),

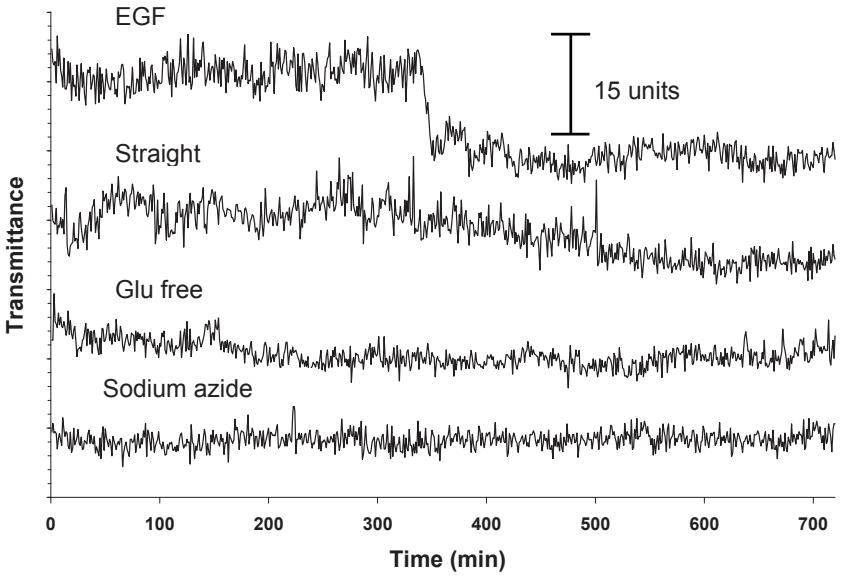

Figure 4. Examples of experimentally-induced changes in photon emission over time after removal from incubation for cells treated with EGF or sodium azide compared to no treatment (straight) and those without glutamine (Glu free). The depression photon emission from the sodium azide and Glu free cells are apparent compared to the untreated cells. Note the sudden (and very replicable) sudden drop in photon emission after 400 min following a protracted elevation in the EGF treated cells.

and whole organisms (Inaba 1988) have been measured for decades. Recently the role of biophotons in the intracellular and intercellular exchange of information or "signals" for communication (Sun et al. 2010) has been supported by direct measurements. In the present study we measured the photon emission from cell cultures that had been removed from their incubated environment for 12 h while and after they cooled to ambient temperatures (21°C). We had reasoned that during periods of perturbations or shifts in physiological states ("cellular stress") there would be greater probability of cell-to-cell or even whole cell culture communication and hence photon emissions would be more likely.

Comparisons of nine different cell types from our library indicated that the power spectra of photon emissions were complex and relatively specific to cells types. Such complexity might be expected if they reflected the membrane's diverse responses to biomolecular changes following removal from incubation. The B16 mouse melanoma cells displayed the most conspicuous power values that were at least twice as high as those displayed by other cells which gave a greater range to study experimental manipulations of the potential sources of the photon emissions.

Various compounds that we selected to explore the source of the photon emissions were given one hour before removal from incubation. Mitochondrial processes, intermediary cytoplasmic pathways, and the plasma cell membrane were primarily loci for consideration. Considering the magnitude of the energy measured which was equivalent to about 10^{-20} J *per* cell a plasma membrane source is most probable (Persinger 2010). Energy generated from glucose- or equivalent-based metabolic activity is in the order of a 10^{-12} J. Both the cell and mitochondria are defined by a membrane with similar order of magnitude discrete potential differences.

The surprisingly distinct increase in energy emission following treatment with EGF for three hours followed by a conspicuous drop in output for the remaining 9 h was considered revealing because the total output for the 12 h did not different from untreated (straight) cells. These two observations strongly suggested the photon source was quantitatively limited and discrete. These characteristics would be reflective of membrane potentials although other candidates could be considered.

EGF receptors are transmembrane proteins that activate a variety of cytoplasmic pathways such as RAS, 3PKC, MAPK, and PKC through cross phosphyloration at the cytoplasmic boundary. The reduction of energy emission by a factor of about five (Table 1) by pretreatment with wortmanin, PD, and BIS (inhibitors of PI3 kinase), MAPK, and PKC would be consistent with the important role of EGF in our measurements. The quantitative outputs did not differ significantly from pretreatment with NaN_3 or the effects of a Glu-free environment. In other words removing energetic sources or inhibiting cytochrome *c* oxidase (Sato et al. 2008) produced a similar diminishment of

photon emission. In addition, after EGF treatment of cells activation EGF receptors not only could activate a variety of cytoplasmic pathways such as RAS, 3PKC, MAPK, and PKC, but also activate free radical producing processes (redox regulation processes). EGF induces NADPH oxidase activity (superoxide generation) and this activity is required for VEGF-mediated migration and proliferation of endothelial cells (Colavitti et al. 2002; Abid et al. 2007). Thus, it is very possible that increase of biophoton intensity after EGF treatment of cells is due to the plasma membrane NADPH oxidase-related enhancement of free radical (superoxide) generation.

In the balance of probabilities the photon emissions were derived from plasma membrane, although we cannot completely exclude contribution from a mitochondrial source. In other experiments we have found that injection of 1 M or 2 M (0.4 cc into the 2.5 cc of the volume containing the cells) KCl produced photon emissions equivalent to the sum of 10^{-20} J for a million melanoma cells. The duration of this effect occurred within about 70 s and 45 s, respectively, after the injection. There was no response with subsequent injections of KCl. These results appear to emphasize the plasma cell membrane more than the mitochondria.

We suggest that the EGF, after being sequestered globally by the 10^5 to 10^6 surface receptors, initiated a process that produced a rapid change in membrane potential within 3 h. On the other hand, pretreatment with ionomycin resulted in more insidious production of membrane apertures through generalized hydrolysis of phosphoionositides within the membrane (Chatilla et al. 1989). The gradual diminishment of the membrane potential would be slower and continuous over 12 h but would be expected to produce greater photon emission.

The cell cycle observations are also consistent with a membrane source to the photon emissions. Time-measurements with respect to cell cycle showed a doubling of photon emission after cell division when there would be approximately twice the number of new cells with stabilizing membrane processes. The approximately doubling of the output at different times during the 12 h of observation following removal from incubation in our sample while their yoked controls were maintained in standard conditions also reduces the probability the photon emissions were spurious phenomena.

From a cytological perspective the observation that the B16 cells' membranes were still intact, according to traditional staining criteria, although either depolarized or near complete depolarization as reflected by intraplasmic indicators, was surprising. Our unpublished measurements indicate indicators of cell death only become discriminable, and then only sparsely so, 24 h after removal from the incubator. These observations indicate that some interesting phenomena might be observed when interventions to "reconstitute" the cells are implemented and photon emission is recorded.

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