Characterization of Calcium Oscillations in Normal and Benzo[a]pyrene-Treated Clone 9 Cells

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Received February 8, 2002; accepted April 15, 2002

Intracellular Ca\(^{2+}\) oscillations induced by oxytocin and vasopressin were analyzed in a rat liver cell line (Clone 9) in order to identify mechanisms by which benzo[a]pyrene (BaP) alters Ca\(^{2+}\) signaling patterns in these cells. Clone 9 cells exhibit an initial Ca\(^{2+}\) spike, followed by Ca\(^{2+}\) oscillations upon oxytocin or vasopressin treatment. The range of frequencies (maximum 110 mHz) was dependent on agonist concentration with a constant amplitude less than or equal to the amount of Ca\(^{2+}\) generated from the inositol trisphosphate (InsP\(_3\))-sensitive pool. This study examined contributions of extracellular and intracellular pools to the frequency of Ca\(^{2+}\) oscillations and the role of membrane channels, second messengers, and different pharmacological reagents on the regulation of oscillation frequency in both control and BaP-treated cells. Results indicated that the Ca\(^{2+}\) oscillations are mainly due to inositol 1,4,5-trisphosphate (InsP\(_3\))-sensitive stores and that extracellular Ca\(^{2+}\) contributes to refilling of this intracellular Ca\(^{2+}\) pool. The frequency of Ca\(^{2+}\) oscillations is also sharply affected by protein kinase C activated by phospholipase C. In BaP-treated Clone 9 cells, basal Ca\(^{2+}\) levels were elevated and the frequency of Ca\(^{2+}\) oscillations was suppressed in a dose-dependent fashion. Suppression of Ca\(^{2+}\) oscillations is due, at least in part, to an effect of BaP on enhanced opening of Ca\(^{2+}\) channels. This was confirmed by showing that inhibition of the K\(^+\) channel opening by tetraethylammonium chloride can reverse the effect of BaP on oxytocin-induced Ca\(^{2+}\) oscillations, and potentially decrease the toxicity of BaP.

Key Words: Ca\(^{2+}\) oscillations; oxytocin; vasopressin; fluorescence microscopy; laser cytometry; BaP.

Calcium signaling in many excitable and nonexcitable cell types frequently occurs as repetitive increases in cytoplasmic-free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)\(_i\)]) referred to as Ca\(^{2+}\) oscillations. The periodic [Ca\(^{2+}\)\(_i\)], spikes, which increase with increasing agonist concentration, are thought to constitute a frequency-encoded signal with a high signal-to-noise ratio, which limits prolonged exposure of cells to high [Ca\(^{2+}\)\(_i\)]. (Sneyd et al., 1995). Analysis of mechanisms that underlie the spatiotemporal patterns of [Ca\(^{2+}\)\(_i\)], has led to an understanding of the role of plasma membrane Ca\(^{2+}\) channels in triggering external Ca\(^{2+}\) influx and inositol 1,4,5-trisphosphate (InsP\(_3\)) receptors and/or ryanodine receptors in the release of Ca\(^{2+}\) from intracellular stores (Berridge, 1997; Berridge et al., 2000; Putney and Bird, 1993).

Ca\(^{2+}\) oscillations appear to be a fundamental mechanism of cell signaling. In systems such as pancreatic acinar cells and pituitary gonadotrophs, where agonists induce oscillatory changes in [Ca\(^{2+}\)\(_i\)], and also stimulate maximal secretion, it appears that frequency-encoded Ca\(^{2+}\) signals exert a physiological role (Stauffer et al., 1993; Stojilkovic et al., 1994; Tse et al., 1993). A number of cellular targets have been identified that are involved in the decoding of these Ca\(^{2+}\) signals. The activity of calcium-calmodulin kinase II (CAM Kinase II) and protein kinase C (PKC) are tuned to the frequency of Ca\(^{2+}\) oscillations (De Koninck and Schulman, 1998; Oancea and Meyer, 1998). Similarly, the activities of Ca\(^{2+}\)-sensitive mitochondrial dehydrogenases in hepatocytes that drive ATP production are controlled by Ca\(^{2+}\) oscillations (Hajnoczy et al., 1995). The selective activation of different transcription factors that can fine tune gene expression appear to be regulated by both amplitude and frequency encoded Ca\(^{2+}\) signals (Dolmetsch et al., 1997; Hu et al., 1999; Li et al., 1998).

In earlier studies, a communication-competent rat liver cell line (Clone 9), which exhibited a complex pattern of oxytocin-induced Ca\(^{2+}\) oscillations, was examined as a potential model to explore effects of selected toxicants on cellular Ca\(^{2+}\) homeostasis (Barhoumi et al., 1996, 2000). The cell line exhibits uniform properties and is widely used for in vitro toxicity studies ranging from oxidative injury (Grune et al., 1995) to chemical carcinogenesis (Na et al., 1995). Like normal liver cells (Arino et al., 1989; Thomas et al., 1995) Clone 9 cells respond to hormones such as oxytocin and vasopressin, which are important glycogenolytic effectors in liver cells and exhibit induction of microsomal ethoxyresorufin-O-deethylase (EROD) activity by BaP (Barhoumi et al., 2000). Oxytocin and vasopressin act primarily by the G protein-coupled formation of InsP\(_3\), via the phosphoinositide-specific phospholipase C

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(PLC) and the subsequent release of [Ca\(^{2+}\)], from intracellular stores (Zingg, 1996). Exposure of these cells to the carciño
genic polycyclic aromatic hydrocarbon (PAH), benzo[a]pyrene (BaP) was found to suppress gap junction-mediated intercel
lar communication (GJIC) and plasma membrane potential, while elevating basal [Ca\(^{2+}\)], (Barhoumi et al., 2000). Given
the diversity of cellular functions that are regulated by [Ca\(^{2+}\)], and the contributions of plasma membrane potential and GJIC
to the regulation and propagation of Ca\(^{2+}\) signals, the present study was designed to investigate the actions of BaP on oxy-
tocin- and vasopressin-induced Ca\(^{2+}\) oscillations in Clone 9 cells. The objectives of this study were to: (1) characterize the
stable, long-term, agonist-induced Ca\(^{2+}\) oscillations in Clone 9 cells; (2) investigate the mechanisms by which BaP may affect
agonist-induced Ca\(^{2+}\) oscillations; and (3) identify experiment-
tal treatments that may partially reverse the effects of BaP.

MATERIALS AND METHODS

Materials. Culture media, Dulbecco’s phosphate-buffered saline (PBS), serum, nifedipine, thapsigargin, oxytocin, vasopressin, ethylene glycol-O,O’-
bis-(β-aminoethyl ether) N,N,N’-tetraacetic acid (EGTA), tetrathylammo-
nium chloride (TEA), 8-bromo-cyclic adenosine-3’5’-monophosphoric acid
(8-Br-cAMP), 8-bromo-cyclic guanosine-3’5’-monophosphoric acid (8-Br-
cGMP) and all general chemical reagents were purchased from Sigma Chem-
ical Co. (St. Louis, MO). Chelerythrine chloride was purchased from A.G.
Scientific, Inc. (San Diego, CA). Tissue culture flasks were obtained from
Corning (Oneonta, NY) and 2-well Lab-Tek Chambered Coverglass slides
were purchased from Nunc, Inc. (Naperville, IL). Fluo-4, AM and the Calcium
Calibration Buffer kit #2 were purchased from Molecular Probes, Inc. (Eugene,
OR).

Stock solution of 1.0 mM fluo-4, AM was prepared in DMSO and diluted
with medium to 3.0 μM (0.3% final DMSO concentration) for loading in
cultured cells. Ten mM nifedipine stock was prepared in DMSO and a 10 μM
concentration was used for experiments. Thapsigargin stock (1.0 mM) was
prepared in DMSO and used at concentrations of 50 nM to 1.0 μM (± 0.1% DMSO).
Chelerythrine chloride was prepared as 10 mM stock in DMSO. Ten mM stock solutions of 8-Br-cAMP and 8-Br-cGMP were prepared in PBS
and used at concentrations of 10 μM to 1.0 mM. TEA was prepared as a 1.0 M
stock in PBS and used at concentrations ranging from 1.0 to 100 mM for cell
treatments. Clone 9 (ATCC, CRL 1439) rat liver cells were obtained at passage
17 and used between passages 25 to 35. Cells were grown in Ham’s Nutrient
Mixture F-12 with 10% fetal bovine serum on 2-well Lab-Tek Chambered
Coverglass slides following plating at a density of 10\(^{6}\) cells per well for 48 h
prior to analysis. To study the effects of BaP on Ca\(^{2+}\) oscillations, actively
growing Clone 9 cells were treated with graded concentrations of BaP (0–20
μM) in the same culture medium for 24 h prior to analysis of [Ca\(^{2+}\)], when
cultures were approximately 80% confluent.

Methods. Fluoro-4, AM, used to monitor [Ca\(^{2+}\)], is a nonratiometric visible
wavelength probe that exhibits about a 40-fold enhancement of fluorescence
intensity with minimal cellular photobleaching. Fluorescence was gener-
ated in the cells by excitation at 488 nm, and fluorescence emission from
scanned individual cells was collected (530 nm) by means of a photomultiplier
tube with a 3-s sampling interval. The basal [Ca\(^{2+}\)], was determined prior to
addition of the hormones and/or pharmacologic agents and fluorescence in-
tensity changes were normalized to this basal [Ca\(^{2+}\)]. Levels, Ca\(^{2+}\) oscillations
were analyzed between 15 and 30 min of addition of hormones when oscilla-
tions were highly uniform. Calibration of intracellular [Ca\(^{2+}\)], was performed as
described by Tsien (1989) using the Calcium Calibration Buffer kit #2 (Molecular Probes).

To determine the source of Ca\(^{2+}\) pools involved in oscillations and their
responding input to the frequency, a variety of pharmacologic agents were
employed. Thapsigargin is an inhibitor of the microsomal Ca\(^{2+}\)-ATPase pump,
which causes leakage of Ca\(^{2+}\) from intracellular InsP\(_3\)-sensitive stores and
prevents their refill (Lyton et al., 1991). It is also used as an indicator for
capacitative Ca\(^{2+}\) entry (e.g., Broad et al., 2001). Chelerythrine chloride is a
potent and selective inhibitor of group A and B PKC isoforms (Herbert et al.,
1990). Both 8-Br-cAMP and 8-Br-cGMP are second messengers that can
influence [Ca\(^{2+}\)]\(_{i}\), by acting on both Ca\(^{2+}\)\(_{i}\) and K\(^{+}\) channels and pumps (Berridge et al.,
2000). TEA is an inhibitor of all known K\(^{+}\) channels (Hille, 1992).

To test each of these agents, cells loaded with fluo-4 were placed on the
Ultima confocal microscope stage and basal fluorescence intensity was ob-
tained from 5 image scans recorded from about 10–15 cells. Following the fifth
scan, cells were exposed to vasopressin or oxytocin and scanning continued at
the same sampling interval until the cells established a uniform pattern of
oscillation (approximately 15 min). Oscillation frequency was then determined
over the next 7 min of the Ca\(^{2+}\) response, a pharmacologic agent was added,
and the frequency was then determined for a similar time interval (Burghardt et al., 1999). Control experiments to monitor any effects of solvent for each
test substance were performed similarly. For each experiment, data were
recorded from 10–15 cells per well of the culture dish and 2–4 wells were
analyzed. Two replicate experiments on consecutive days were performed.

Ca\(^{2+}\) oscillation frequency data are expressed as means ± SEM mHz.
Differences between treatments performed on the same day were evaluated by
ANOVA, followed by Dunnett’s or Tukey’s test for multiple comparisons at
p < 0.05.

RESULTS

Characterization of oxytocin- and vasopressin-induced Ca\(^{2+}\) oscillations in normal and BaP-treated Clone 9 cells. Oxy-
tocin and vasopressin were used to induce Ca\(^{2+}\) oscillations in
Clone 9 cells, examples of which are shown in Figure 1. Uniform oscillations were established within 15 min of addi-
tion of hormone and remained uniform for at least 30 min. The

![Graph showing Ca\(^{2+}\) oscillations in BaP-treated Clone 9 cells](image_url)
frequency of these oscillations was concentration-dependent, although Clone 9 cells were more sensitive to vasopressin than to oxytocin (Fig. 2). For example, analysis of data obtained from individual cells revealed that the average frequency of oscillation in control Clone 9 cells treated with 100 nM oxytocin became stable with a value of 47.25 ± 3.55 mHz (i.e., one oscillation every 21 s) when Ca$^{2+}$ was present in the culture medium. The maximum increase in the oscillating Ca$^{2+}$ signal was approximately 136 nM above basal [Ca$^{2+}$], represented by a normalized value of about twice the basal [Ca$^{2+}$], level detected prior to initiation of oscillations (Fig. 1). The steady state [Ca$^{2+}$], about which cells oscillate, did not change from the basal [Ca$^{2+}$], detected prior to addition of oxytocin. However, stable oscillations with an average frequency of 94.56 ± 8.27 mHz to 24.22 ± 7.96 mHz within the same time interval. One μM thapsigargin completely eliminated all agonist-induced Ca$^{2+}$ oscillations. The magnitude of the Ca$^{2+}$ peak during thapsigargin treatment was equal to or greater than the peak of Ca$^{2+}$ oscillation induced by hormone alone (data not shown). Cells exposed to 10 μM BaP exhibited increased sensitivity to thapsigargin as addition of 50 nM of this microsomal Ca$^{2+}$-ATPase pump inhibitor to cultures with ongoing 100 nM oxytocin- or 0.5 nM vasopressin-induced Ca$^{2+}$ oscillations completely eliminated all oscillations.

**Regulation of Ca$^{2+}$ oscillations in normal and BaP-treated Clone 9 cells by protein kinase C, second messengers, and K$^+$ channels.** To study the contribution of PKC, and the cGMP- and cAMP-second messenger pathways to the frequency of Ca$^{2+}$ oscillations in control and BaP-treated Clone 9 cells, the PKC inhibitor chelerythrine chloride and second messenger analogs (8-Br-cGMP and 8-Br-cAMP) were used. Rapid, dose-dependent suppression of oxytocin-induced Ca$^{2+}$ oscillations was recorded with each reagent, although the relative sensitivity of the oscillations to each reagent differed (Fig. 4). Addition of chelerythrine chloride (PKC inhibitor), 8-Br-cGMP or 8-Br-cAMP to BaP-treated cells exhibited a similar response to control Clone 9 cells (data not shown).

To further investigate the basis for the earlier observation of suppression of plasma membrane potential by BaP (Barhoumi et al., 2000), the action of the K$^+$ channel blocker, TEA, was examined. Addition of TEA did not significantly alter the frequency of agonist-induced Ca$^{2+}$ oscillations in untreated
Clone 9 cells. In contrast, addition of the K⁺ channel blocker, TEA (10 mM) to the ongoing oxytocin- or vasopressin-induced Ca²⁺ oscillations restored the amplitude as well as the frequency of Ca²⁺ oscillations in BaP-treated cells to that of control levels. To examine the responses of individual cells in BaP-treated cultures to TEA, a subset of cells that exhibited loss of oscillatory behavior were examined. An example of the restoration of Ca²⁺ oscillations caused by addition of TEA is shown in Figure 5, and quantitative assessment of these cells is shown in Figure 6. These data suggest that the open state of K⁺ channels in Clone 9 cells exposed to BaP is enhanced resulting in suppression of Ca²⁺ oscillations. Further, restoration of Ca²⁺ oscillations can be achieved by reversing the open state of these plasma membrane channels.

**DISCUSSION**

Free [Ca²⁺], is a versatile and universal signaling agent that plays a pivotal role in cellular homeostasis in virtually every
cell type, e.g., as a second messenger involved in signal transduction, and as point of convergence for other second messenger pathways linking events initiated at the cell membrane to biological responses. Intracellular \(Ca^{2+}\) is consequently in a position to accumulate and integrate information from multiple signaling systems and to convert information into a form or code that regulates downstream events ranging from sequestration to gene expression. The existence of diverse \(Ca^{2+}\) signaling patterns that vary in frequency and/or amplitude has led to the concept that many \(Ca^{2+}\)-regulated processes are controlled by these codes (Berridge, 1997). A number of cellular and mitochondrial enzymes and transcription factors have been identified as decoders of \(Ca^{2+}\) signals. For example, CaM kinase II can discriminate between low and high frequency \(Ca^{2+}\) signals by decoding \(Ca^{2+}\) oscillations into graded levels of kinase activity (De Koninck and Schulman, 1998). Other decoders include certain isoforms of PKC, PLC, mitochondrial dehydrogenases and transcription factors including CREB, CREM, NF-AT, and NF-kB (Dale et al., 2001; Dolmetsch et al., 1997; Hajnoczky et al., 1995; Hardingham et al., 2001; Hu et al., 1999; Oancea and Meyer, 1998). In the case of \(Ca^{2+}\)-sensitive transcription factors, numerous target genes are affected.

In the present study, analysis of the frequency of \(Ca^{2+}\) oscillations was used to characterize the dose-dependence and cytoplasmic mechanisms controlling oxytocin- and vasopressin-induced frequency encoded \(Ca^{2+}\) signals in an immortalized rat liver cell line and to determine the effects of BaP on these processes. Both oxytocin and vasopressin induce uniform, stable oscillations and the frequencies of these oscillations are concentration-dependent, with vasopressin having a steeper concentration-frequency relationship, greater potency, and maximum effect. The highest frequencies obtained for oxytocin and vasopressin were 72.4 ± 4.0 mHz and 109.5 ± 7.1 mHz, respectively. Differences between hormone-induced frequencies may be due to the number of receptors and/or the relative affinity of the receptors for these vasoactive hormones with actions on glycogenolysis and mitochondrial energy metabolism in liver (Hajnoczky et al., 1995; Zingg, 1996).

The main intracellular source of \(Ca^{2+}\) involved in these oscillations is the InsP3-sensitive pool activated through stimulation of PLC-\(\beta\) via G-protein-linked receptors. The presence of extracellular \(Ca^{2+}\) is required to maintain the oscillations, indicating the importance of plasma membrane \(Ca^{2+}\) fluxes in refilling the intracellular pools. Activation of PKC as a result of PLC-\(\beta\) activation constitutes a potential inhibitory feedback loop on this pathway that may contribute to \(Ca^{2+}\) oscillations. Using the PKC inhibitor, chelerythrine chloride, the frequency of oxytocin-induced \(Ca^{2+}\) oscillations dropped at a high rate; oscillations were rapidly abolished at 20 \(\muM\). These data suggest the importance of the PKC feedback mechanism in controlling \(Ca^{2+}\) oscillations in Clone 9 cells where PKC is repetitively activated by \(Ca^{2+}\) and diacylglycerol as the result of PLC-\(\beta\) activation to terminate individual \(Ca^{2+}\) spikes (Codazzi et al., 2001). This is accomplished by PKC phosphorylation of oxytocin or vasopressin receptors to uncouple them from activation of Gq and/or phosphorylation of PLC-\(\beta\) and prevent its activation by Gq (Taylor and Thorn, 2001).

The frequency of \(Ca^{2+}\) oscillations in Clone 9 cells is also influenced by cytoplasmic levels of cAMP and cGMP, since both 8-Br-cAMP and 8-Br-cGMP cause a dose-dependent suppression of oscillations. The specific targets of these second messengers in Clone 9 cells, which modulate oscillation frequency, have not been defined; however, there are numerous ways in which their effects can be mediated. Both 8-Br-cAMP and 8-Br-cGMP can influence \([Ca^{2+}]_{i}\) by acting on both \(Ca^{2+}\) channels and pumps (Berridge et al., 2000). \(Ca^{2+}\)-activated K+ channels can be stimulated by both cAMP and activation of soluble guanylate cyclase to oppose depolarization stimuli (Berridge et al., 2000; Lee and Kang, 2001) and cAMP can inhibit oxytocin-induced phosphatidyl inositol turnover and increases in \([Ca^{2+}]_{i}\), through protein kinase A-mediated phosphorylation of certain isoforms of PLC (Yue et al., 2000).

In this investigation, use of \(Ca^{2+}\) oscillation frequency analysis was useful to better define the adverse effects of BaP on intracellular \(Ca^{2+}\) homeostasis and signaling. We previously reported the rapid uptake and partitioning of BaP into cellular membranes that resulted in uncoupling of gap junctions, increases in \([Ca^{2+}]_{i}\), and hyperpolarization of cells (Barhoumi et al., 2000). These effects suggested a direct action of BaP on multiple membrane targets which could alter signaling mechanisms involving \([Ca^{2+}]_{i}\), as a second messenger. The present studies confirm this adverse effect of BaP resulting from suppression of oxytocin- and vasopressin-induced frequency-encoded \(Ca^{2+}\) signals. Treatment of Clone 9 cells with 10 \(\muM\) BaP for 24 h altered the frequency response for both hormones with a significantly greater impact on vasopressin-induced \(Ca^{2+}\) oscillations. This agonist-specific impact of BaP may be due to the different dose frequency and amplitude patterns of \(Ca^{2+}\) oscillations exhibited by both hormones in control Clone 9 cells.

The contribution of PKC to the frequency of \(Ca^{2+}\) oscillation did not change in BaP-treated cells as the PKC inhibitor reduced the frequency at the same rate of control cells. Both 8-Br-cGMP and 8-Br-cAMP had the same effect on frequency in BaP-treated cells when compared to control cells, which indicates that PKC, cGMP, and cAMP pathways were not detectably altered by BaP treatments.

The effects of BaP were noticeable on the pools involved in determining the initial \(Ca^{2+}\) transient and the frequency of \(Ca^{2+}\) oscillations. These effects may be due in part to the increase in basal \([Ca^{2+}]_{i}\), caused by a direct action of BaP on \(Ca^{2+}\) and/or K+ channels. Analysis of the initial \(Ca^{2+}\) transients induced by oxytocin identified no significant effects of BaP on voltage-operated \(Ca^{2+}\) channels (nifedipine-sensitive), InsP3-sensitive stores, or stores operated \(Ca^{2+}\) channels (thapsigargin-sensitive). However, a direct action of BaP on plasma membrane K+ channels may account for previous observations of hyperpolarization of the plasma membrane within 30 min of addition of
BaP to culture medium (Barhoumi et al., 2000). Direct evidence in support of this suggestion was provided in the present study by the action of the K⁺ channel blocker, TEA, which reestablished a uniform pattern of Ca²⁺ oscillations with a frequency comparable to that in control cells, by partial cellular depolarization and possibly by mediating the sequestration of Ca²⁺ into intracellular stores. Collectively, these studies suggest that membrane channels, including gap junctions and K⁺ channels, are important targets of BaP toxicity. If BaP exerts similar effects in vivo, the consequence may be impairment of tissue homeostasis and function.

It is noteworthy that alterations of Ca²⁺ homeostasis in cells exposed to PAHs have been most extensively investigated in other persistent environmental pollutants such as polychlorinated biphenyls (PCBs). Among PCBs, perturbation of Ca²⁺ signaling appears to be greatest among the noncoplanar, non-Ah receptor-activating, ortho-substituted PCB congeners (Kodavanti and Tilson, 2000). These nondioxin-like PCBs accumulate in the brain and can interfere with Ca²⁺ homeostasis via multiple targets, including ryanodine receptors (Wong et al., 1997). L-type and/or voltage-operated Ca²⁺ channels (Bae et al., 1999), and Ca²⁺ buffering systems (Yang and Kodavanti, 2001) leading to translocation of PKC.

In contrast, the actions of dioxin-like PAHs on Ca²⁺ homeostasis appear to be subtler. BaP and 2,3,7,8-tetrachlorodibenzo-p-dioxin can induce sustained elevation of Ca²⁺, which is thought to involve cytochrome P450-mediated metabolism (Hanneman et al., 1993; Romero et al., 1997; Tannheimer et al., 1999). BaP metabolites were more effective in elevating Ca²⁺ than the parent compound (Mounho and Burchiel, 1998; Romero et al., 1997; Tannheimer et al., 1999). Oxidative metabolism of BaP can result in redox stress, which could alter voltage-sensitive pathways in membranes. Similar suppressive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on Ca²⁺ oscillations in Clone 9 cells have been observed, whereas the weak ary1 hydrocarbon agonist 1,2,3,4-TCDD had no effect on oscillations (Barhoumi et al., 1999; Mouneimne et al., 1999). Further studies are needed to clarify how BaP modulation of redox pathways in cells may affect K⁺ channel activity in Clone 9 cells. It is also possible that alterations in Ca²⁺ homeostasis in Clone 9 cells observed in the present study may involve cytochrome P450-independent mechanisms. It has been reported that BaP-induced alterations in signal transduction pathways may contribute to tumor promotion and progression through nongenotoxic mechanisms (Parrish et al., 1998).

Given the importance of [Ca²⁺], in signal transduction, cellular proliferation, mitochondrial energy production, and gene expression, a better understanding of (1) how the conversion of signal transduction events initiated at the cell surface are converted into code that regulates downstream events, and (2) what the consequences are of toxicant-altered, encoded Ca²⁺ signals. The present studies provide a powerful experimental approach to begin to address these issues. Studies are currently underway to define the functional consequences of BaP-altered amplitude and frequency-encoded Ca²⁺ signals.

ACKNOWLEDGMENTS

This work was supported in part by NIH grants P42-ES04917 and P30-ES09106 ES and funding from the Texas Agricultural Experiment Station.

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