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TITLE: ELEVATED CYTOSOLIC Ca^{2+} DOES NOT MEDIATE CELL DEATH FROM CHEMICAL ANOXIA IN A NEURONAL CELL LINE

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Elevated ionized cytosolic Ca^{2+} (Ca^{2+}_i) has been implicated as a mechanism of anoxic cell death in neurons. However, in hepatocytes Ca^{2+}_i is not involved in anoxic death.[1] Thus, our aim was to measure, at the level of the single cell, the relationship between Ca^{2+}_i and cell death during ATP depletion in a cultured cell line of neuronal origin, SK-N-SH, derived from human neuroblastoma.

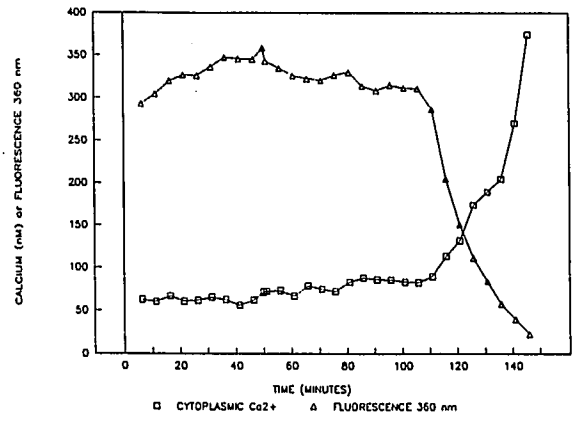
METHODS.[1,2] Cells cultured on coverslips were loaded with the fluorescent Ca^{2+}_i indicator fura-2 (30 min, 37°C, 5 μM AM ester), and placed in HEPES-supplemented Krebs-Ringer buffer (2 mM $CaCl_2$, pH 7.4). Fluorescence of single cells was monitored using excitation at 340 and 360 nm, and digitized video fluorescence microscopy.[2] Ca^{2+}_i was calculated from the ratio of fluorescence at 340 nm (Ca-sensitive) and 360 nm (Ca-insensitive) of cells compared to known standards. The onset of cell death was determined from the loss of fluorescence at 360 nm.

RESULTS. Cytoplasmic localization of de-esterified fura-2 was shown to be virtually 100% by complete loss of fluorescence at 360 nm with 20 μM digitonin. Cells responded to acetylcholine with a 4-fold increase in Ca^{2+}_i, as expected for neurons.

Initial [Ca^{2+}_i] was 118 ± 52 nM (n = 11 cells). Chemical anoxia with 2.5 mM NaCN and 10 mM 2-deoxyglucose was used to mimic the ATP depletion of anoxia. Time to cell death at 37°C was 45 ± 48 min. Ca^{2+}_i changed minimally during anoxia until the onset of cell death ($\Delta[Ca^{2+}_i]$ = 11 ± 19 nM). The only major elevation of Ca^{2+}_i occurred simultaneously with a precipitous decline in 360 nm fluorescence at the time of cell death.

CONCLUSION. Elevated Ca^{2+}_i does not contribute to neuronal cell death during chemical anoxia. An abrupt increase in Ca^{2+}_i occurs only as a terminal event associated with the loss of cell membrane integrity and the influx of Ca^{2+} along its concentration gradient.

REFERENCES. 1) Cell Calcium 9:237, 1988. 2) Cell Calcium 11:63, 1990.



Typical response of single SK-N-SH cell to chemical anoxia, begun at 49.9 min. Cell death is indicated by the sudden decrease in 360 nm fluorescence.

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TITLE: A COMPARATIVE ANALYSIS OF ENFLURANE DOSE-EFFECT ON PRIMATE MOTOR AND SOMATOSENSORY EVOKED POTENTIALS

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Combined motor (MEP) and somatosensory (SEP) evoked potential recordings under anesthesia is highly desirable particularly in major spinal cord surgery. The influence of enflurane on both modalities was examined.

Cranial (Cr) and peripheral (Pr) MEPs, in response to 1.5 Tesla pulsed magnetic fields applied respectively to the scalp[1] and cervical and lumbosacral spine, were recorded from abductor pollicis brevis and abductor hallucis muscles. Scalp sub-(N1), early-(N2), middle-(N3) and late-(N4) cortical SEPs, in response to contralateral median (MN) and posterior tibial (PTN) nerve stimulation at the wrist and ankle were recorded. After obtaining MEP-SEP baseline, 10 monkeys were allowed to inhale 0.25, 0.5, 0.75 and 1.0 MAC endtidal enflurane via endotracheal tube. The dose-response curve of enflurane and MEP-SEP variables were studied using one-way ANOVA.

Inhalation of $\geq$ 0.25 MAC enflurane caused immediate substantial degradation of Cr-MEPs: shrinkage of MEP scalp fields, stimulation threshold rise, amplitude depression, and response obliteration (>0.5 MAC). Replicable but attenuated Pr-MEPs and SEPs were obtained. (Figure 1)

The results indicate that enflurane (a) has a differential effect on MEPs and SEPs, the former being extremely vulnerable; (b) suppresses motoneurones at subanesthetic doses; and (c) inhibits MEP, chiefly through central mechanisms. Avoidance of volatile anesthetics may be advantageous while monitoring MEP.

References.
1. J Neurosurg Anesthesiol 2:79-85, 1990

FIG. 1.