



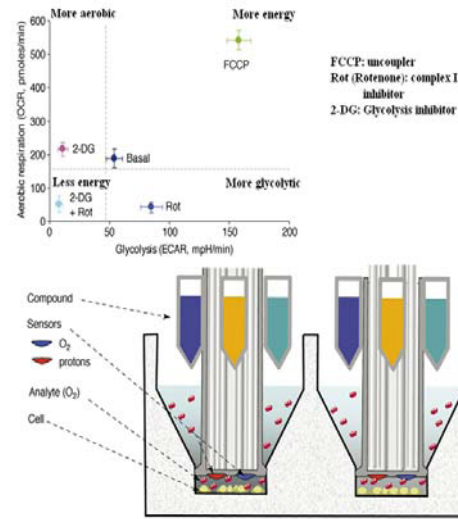
ABRUPT ALCOHOL WITHDRAWAL SUPPRESSES MITOCHONDRIAL RESPIRATION IN AN ESTROGEN PREVENTABLE MANNER.

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ABSTRACT

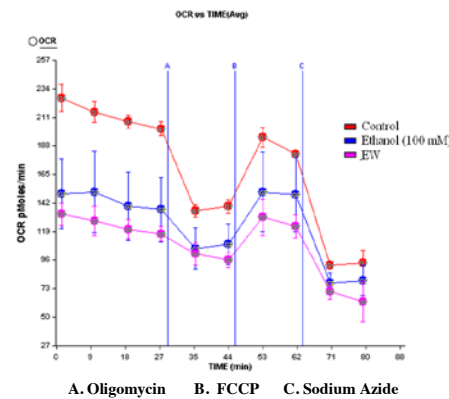
We previously demonstrated that abrupt ethanol withdrawal (EW) inhibits a key mitochondrial enzyme cytochrome c oxidase (COX, i.e. mitochondrial complex IV) and 17-beta estradiol (E2) mitigates this problem. Here, we investigated whether EW suppresses mitochondrial respiration through inhibition of COX in a manner protected by E2. To do this, we measured mitochondrial O2 consumption rate (OCR) and glycolysis by monitoring extracellular acidification rate (ECAR) using XF24 analyzer. HT22 cells were exposed to ethanol (100 mM) for 72 hrs and withdrew for 4 hrs. E2 (10 microM) was administered either during ethanol exposure or EW phase. A COX inhibitor (NaN3) at the dose of 1 or 5 mM was injected while XF analyzer began to read OCR. We found that compared to a control condition (non-ethanol), EW suppressed the basal level of OCR more severely than ethanol exposure per se, which was accompanied by ATP depletion and excess glycolysis. Compared to control cells, OCR was suppressed by a lower dose of NaN3 in ethanol withdrawn cells which concurred with cell death. Finally, E2 treatment restricted to the EW phase protected against EW-induced suppression of OCR. These results suggest that EW provokes mitochondrial bioenergetic deficit in a manner protected by estrogen (supported by NIAAA AA013864 and AA015982).



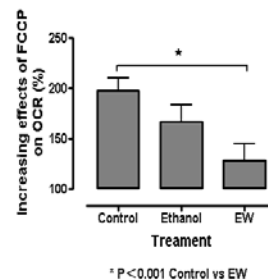
The XF24 analyzer measures the rate of changes in the media surrounding living cells. The measurement is performed using 24 optical fluorescent biosensors embedded in a sterile cartridge that is placed into the XF24 cell culture plate.

RESULTS

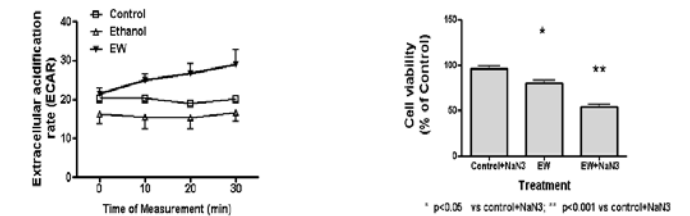
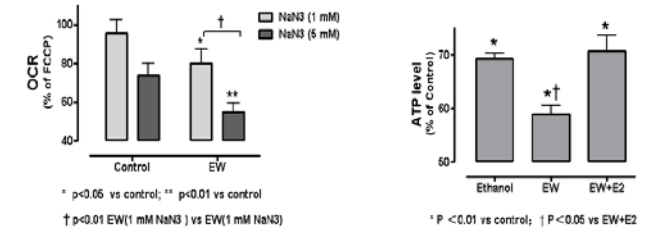
1. Ethanol withdrawal (EW) suppresses O2 consumption rate more severely than Ethanol exposure.



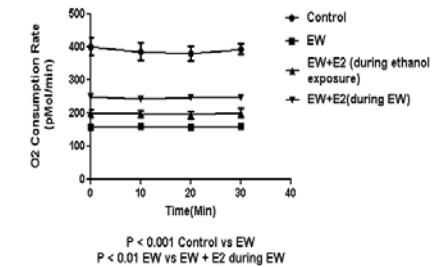
2. Ethanol withdrawal (EW) suppresses maximum respiratory capacity.



3. Ethanol withdrawal (EW) provokes bioenergetic deficit through inhibiting cytochrome c oxidase (COX) that is accompanied by ATP depletion, increased glycolysis, and exacerbated cell death.



4. Estrogen (E2, 10µM) protects against ethanol withdrawal (EW)-induced suppression of oxygen consumption rate.



CONCLUSIONS

Ethanol withdrawal compromises mitochondrial bioenergetic function associated with a key mitochondrial enzyme, cytochrome c oxidase in a manner protected by estrogen.

ACKNOWLEDGEMENT

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MATERIALS AND METHODS

Reagents

17-Estradiol (E2) was purchased from Steraloids (Wilton, NH). Oligomycin, FCCP and sodium azide were purchased from Sigma (Louis, MO). Calcein-AM was purchased from Molecular Probes (Eugene, OR). HT22 cells, a mouse-derived hippocampal cell line, were the generous gift of Dr. David Schubert (Salk Institute, San Diego, CA).

Cell Culture and ethanol treatment

HT22 cells were maintained in Dulbecco's modified Eagle's medium, supplemented with 10% charcoal-stripped fetal bovine serum (HyClone, Logan, UT) and 1% penicillin-streptomycin at 37°C in an atmosphere containing 5% CO2 and 95% air. The HT22 cells were divided into several groups including control group (CTL), ethanol exposure group (EXP), ethanol withdrawal group (EW) and EW/E2 group. HT22 cells in EXP and EW groups were exposed to 100 mM ethanol for 3 days. EW group went through withdrawal for 4 hrs. Regarding EW/E2 group, 10 µM E2 was added during ethanol exposure or EW. In order to keep ethanol concentration the cell plate was placed into a plastic box which contained higher level of ethanol. During EW the media was changed into the one without ethanol.

Calcein-AM Cell Viability Assay

Calcein AM (Calcein acetoxymethyl ester), a non-fluorescent, cell permeable compound, converts to the strongly green fluorescent calcein when hydrolyzed by intracellular esterases in live cells. It is widely used for determining cell viability since the fluorescence intensity of calcein is proportional to the amount of live cells. After the removal of the medium from the 96-well plates, the cells were rinsed once with phosphatebuffered saline (PBS; pH 7.4) and incubated in a solution of 2.5 µM calcein-AM in PBS. Twenty minutes later, fluorescence was determined using a Bio-Tek FL600 microplate reader (Bio-Tek Instruments, Winooski, VT) with an excitation/emission filter set at 485/530 nm.

OCR and ECAR assay

HT22 cells were seeded into XF24 plates at the density of 600/well, and divided into several groups including CTL, EXP, EW and EW/E2 group. HT22 cells were treated as mentioned above. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured at the end of 3-day ethanol exposure and 4-hour EW using XF24 analyzer (Seahorse Bioscience, MA). To characterize the effect of EW on OCR and ECAR, 1.0 µg/ml of oligomycin (the inhibitor of mitochondrial complex V), 0.3 µM FCCP (mitochondria uncoupler) and 1 mM or 5 mM sodium azide (the inhibitor of mitochondria complex IV) were added successively 30 min after the assay began.