Estratiol dependent molecular and biochemical change in human stermatoza

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ABSTRACT

In organotypic hippocampal slice cultures, reducing A-type K1 current (IA) increases NMDAR-mediated EPSCs with no effect on AMPAR-mediated EPSCs (Jung et al., 2008). Interestingly, this effect is specific for NR2B-containing NMDARs. E2 is known to modulate K1 channels in several brain regions (Nabekura et al., 1986; Minami et al., 1990). In the hypothalamus, 1 week of E2 treatment reduced the current density of IA in an ER-dependent manner (DeFazio and Moenter, 2002). If E2 acts similarly in the hippocampus, a reduction in IA might account for the observed increase in NR2B-dependent NMDAR EPSCs in a way that would not require changes in expression levels or phosphorylation.

INTRODUCTION

Estradiol dependent molecular signaling has traditionally been identified with the transcriptional control of target genes via the binding of nuclear Estradiol dependent molecular receptors to genomic consensus sequences. Nonetheless, in the past few years, several biological actions of Estradiol dependent molecular have been identified that are too rapid to be compatible with transcriptional mechanisms.

Rapid induction of no synthesis by Estradiol dependent molecular largely depends on activation of the endothelial is form of NO synthase (eNOS). Estradiol dependent molecular receptor (ER) is involved in this phenomenon, which is in part attributable to mitogen-activated protein (MAP) kinases or tyrosine kinase activation.

The site of interaction between PI3K and ER_ is unclear. Because PI3K is mainly cytoplasmic, it may be possible that cytoplasmic or cell membrane–bound ERs are responsible for the recruitment of PI3K. Indeed, extranuclear ERs have been described long since,18 and, very recently, membrane-bound ERs have been identified in endothelial cells and implicated in the regulation of NO production.

LITERATURE REVIEW

In human endothelial cells, physiological concentrations of 7-estradiol (E2) acutely increase NO release via an ER and PI3K-dependent mechanism.8 Different from insulin dependent activation, type III NOS is recruited in a biphasic manner (EC50 value of _0.1 nmol/L), showing an early induction within 2 minutes, followed by a more substantial increase after 15 to 20 minutes.

Estradiol dependent molecular-response element linked to a luciferase gene, we found that although actinomycin D does not block Estradiol dependent molecular stimulated eNOS activity, it blocks Estradiol dependent molecular-induced gene transcription suggesting that E2 activates eNOS through non-transcriptional mechanisms.

Alternative Explanations In lieu of increasing NMDAR subunit expression levels or altering NR2B phosphorylation, what could account for the effect of E2 to enhance NMDAR EPSCs? As suggested by the results of Jelks et al. (2007), one likely possibility is recruitment of existing NMDARs to synaptic sites. Extrasynaptic NMDARs account for over 35% of total surface NMDAR receptors and may preferentially contain the NR2B subunit (Tovar and Westbrook, 2002; Harris and Pettit, 2007). Although the NMDAR

content of synapses is thought to be relatively stable in adulthood, compared with AMPAR content, it is possible that E2 induces a rearrangement of existing NR2B-containing NMDARs to synapses. One way E2 could effect the localization of NMDARs is by increasing CamKII activity.

OBJECTIVES

Spermatozoa were cultivated in the laboratory at 37°C in incubator. The control group (medium without NP) was compared to the experimental groups (exposed to different concentrations of NP with the addition 17β-estradiol) during 0 h and 6 h of *in vitro* cultivation.

Statistical analysis

Obtained data were statistically analyzed using PC program GraphPad Prism 3.02 (GraphPad Software Incorporated, San Diego, California, USA). Descriptive statistical characteristics (mean, minimum, maximum, standard deviation and coefficient of variation) were evaluated. T-test and Wilcoxon matched pairs test were used for statistical evaluations.

Computer-assisted semen analysis (CASA)

The Olympus BX 51 microscope (Olympus, Tokyo, Japan) at cultivation times 0 h and 6 h. Each sample was placed into the Makler Counting Chamber (deph 10 μm, Sefi-Medical Instruments, Haifa, Izrael) and the following parameters evaluated: the percentage of motile spermatozoa (motility > 5 μm/s; MOT) and the percentage of progressively motile spermatozoa (motility > 20 μm/s; PROG).

RESEARCH METHODOLOGY

Proteasomes have been detected in all eukaryotic cells studied to date, and also in the gametes of numerous species. In sea urchins proteasomes are involved in the acrosome reaction (Matsumura & Aketa, 1991), while in ascidians sperm proteasomes take part in the fertilization process (Saitoh et al., 1993) since proteasome inhibitors block fertilization (Takizawa et al., 1993). Proteasomes are present in salmon spermatozoa along the axoneme (Inaba & Morisawa, 1992; Inaba et al., 1993), and apparently perform some function related to their motility because proteasome inhibitors immobilise the spermatozoa (Inaba et al., 1993).

A double volume of sample buffer was used for each volume of whole semen or seminal while the pellet obtained For motility analysis and immunouorescence labelling, motile spermatozoa were selected by centrifugation through a discontinuous Percoll gradient (Berger et al., 1985). Briey, 1 mL semen was carefully layered over a gradient of Percoll and centrifuged for 20 min at 350 g in a Jouan C1000 S5L centrifuge. The pellet was resuspended in 5 mL Ham's F10 nutrient mixture, and centrifuged again for 10 min at 500 g then resuspended in an appropriate volume of IVF medium (Medicult, Denmark) and used for further procedures.

SUMMARY & CONCLUSION

SDS-PAGE and Western blotting Sperm samples were heated by short immersion in boiling water and run on 12% SDS-PAGE in a vertical slab gel unit (Owl Scienti®c Inc., Woburn, MA, USA) (Laemmli, 1970). After SDS-PAGE the gel was blotted by a semidry method using the isotachoforetic buffer system with The Pantherä electroblotter (Owl Scienti®c Inc., Woburn, MA, USA) on the PVDF transfer membrane (Biorad, Hercules, CA, USA). After transfer, the membrane was blocked (5% dry skimmed milk, 2% BSA, 0.2% Tween-20 in TBS), washed three times, incubated with primary antibody, washed three times again, and incubated with secondary antibody conjugate.

CONCLUSION

Cells were then quenched in 50 mMNH4Cl, permeabilized in 0.1% Triton X-100 and washed twice in TBS (Tris-buffered saline, pH 7.6) supplemented with 1% BSA and 1% skin gelatine, before incubation with the antibody. After extensive washing, the slides were incubated with the secondary antibody and washed in TBS. Slides were treated with RNAse for 15 min and counterstained with propidium iodide or Yo-Proä-1-iodide.

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