Effects of Ubiquitin C-Terminal Hydrolase L1 (UCH-L1) Inhibition on Sperm Incorporation and Cortical Tension in Mouse Eggs

Ubiquitin C-Terminal Hydrolase L1 (UCH-L1) is thought to have multiple functions in mammalian oocytes and early embryos (e.g., Mtango et al., 2011; Sekiguchi et al., 2006). Here we tested the overall hypothesis that UCH-L1 has functions in the mouse egg cortex and/or overlying plasma membrane, given that UCH-L1 is enriched in the egg cortex (Sekiguchi et al., 2006). We examined egg membrane receptivity to sperm by assessing sperm incorporation over time with zona pellucida-free eggs, as previously described (McAvey et al., 2002), in the presence or absence of the UCH-L1 inhibitor LDN-57444, the development and specificity of which was described by Liu et al. (2003). Zona-pellucida-free eggs from superovulated CF-1 female mice were treated with 0, 5, or 10 μM LDN-57444 (Calbiochem, San Diego, CA; stock solution prepared in DMSO) for 2 hr, inseminated with 10^5 sperm/ml, and then examined at 1 and 3 hr post-insemination. Under these conditions, control eggs are fertilized effectively and polyspermy is low (Table 1). In contrast, LDN-57444-treated eggs show an increase in polyspermy and in sperm-egg fusion events over time (Table 1). This suggested that inhibition of UCH-L1 disrupts the ability of the egg membrane to convert to an unreceptive state after fertilization.

The membrane block to polyspermy is mediated in part by the egg cytoskeleton, and experimental manipulations that disrupt establishment of this membrane block also alter tension in the cortical cytoskeleton (McAvey et al., 2002; Larson et al., 2010). We therefore assessed the effects of LDN-57444 on cortical tension in eggs using micropipette aspiration (Larson et al., 2010), a highly sensitive readout of contractility in the cortical cytoskeleton that is regulated by actin, non-muscle myosin-II, and links between actin filaments and to the overlying plasma membrane. LDN-57444-treated eggs had decreased cortical tension as compared to DMSO-treated eggs, with a nearly 50% decrease in the microvillar domain, which supports sperm binding and fusion, and the amicrovillar domain, which sequesters the meiotic spindle (Table 1).

Together, these data indicate that UCH-L1 affects certain features of the egg cortex and plasma membrane. The ubiqutitin system in general, and UCH-L1 specifically, has been implicated in the function of actin-associated processes and structures, including cortical tension (e.g., Bassères et al., 2010; Vergara et al., 2014). The data here support a model that the egg cortex in some way impacts the egg's responsiveness to a fertilizing sperm and/or conversion of the membrane to an unreceptive state after the first sperm has penetrated (McAvey et al., 2002). Although increased polyspermy was not detected in in vivo fertilization studies with one mouse model with a *Uchl1* mutation (Mtango et al., 2011), increased polyspermy was observed following in vitro fertilization of eggs from this mutant model (Sekiguchi et al., 2006), which is consistent with the fact that multiple factors contribute to polyspermy prevention in vivo. The assays used here, including acute pharmacological inhibition of UCH-L1 activity, complement the in vivo, genetic studies using models with chronic UCH-L1 deficiency, and support the use of combined approaches to advance our understanding of reproductive defects. In particular, elucidating factors that contribute to polyspermic fertilization has high relevance to human reproductive health, given that dispermic fertilization occurs in human conceptions in vivo and in vitro (e.g., Zaragoza et al., 2000).

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TABLE 1. Effects of the UCH-L1 Inhibitor LDN-57444 on Sperm Incorporation Over Time Into Zona-Pellucida-Free Eggs, and on Cortical Tension

Endpoint	0 μM (0.025% DMSO)	5 μM LDN-57444	10 μM LDN-57444
% eggs fertilized, 1 hr post-insemination	80%	90%	91%
% eggs fertilized, 3 hr post-insemination	100%	100%	100%
% eggs polyspermic, 1 hr post-insemination	0%	10%	15%*
% eggs polyspermic, 3 hr post-insemination	11%**	70%*,**	100%*,**
% eggs with 3+ sperm fused, 1 hr post-insemination	0%	0%	0%
% eggs with 3+ sperm fused, 3 hr post-insemination	0%	18%*,**	81%*,**
Av. number of sperm fused per egg, ± standard error 1 hr post-insemination	0.80 ± 0.06 sperm/egg	1.00 ± 0.07 sperm/egg	1.03 ± 0.08 sperm/egg
Av. number of sperm fused per egg, ± standard error 3 hr post-insemination	1.11 ± 0.05 sperm/egg	1.88 ± 0.12 sperm/egg*,**	3.19 ± 0.15 sperm/egg*,**
Av. cortical tension ± standard error, microvillar domain (away from the metaphase-II spindle)	0.85 ± 0.02 nN/μm	No data	0.45 ± 0.02 nN/μm*
Av. cortical tension ± standard error, amicrovillar domain (over the metaphase-II spindle)	1.88 ± 0.07 nN/μm	No data	0.97 ± 0.08 nN/μm*

Statistical analyses: Unpaired Student's *t*-test for data on extent of polyspermy; ANOVA with Tukey's post-hoc testing for data on average number of sperm fused per egg; Mann–Whitney *U*-test for data on cortical tension.
*$P < 0.05$ as compared to DMSO-treated eggs.
**$P < 0.05$ as compared to the 1 hr post-insemination time point.

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