D111 Nicotinic Acetylcholine Receptor Induces Calcium Influx in Cultured Chick Myoblast

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Calcium influx appears to be pivotal in triggering myoblast fusion. Previously, we have reported that reciprocal activation of calcium-activated potassium channels (K_{Ca}) and stretch-activated channels induces hyperpolarization of membrane potential and then increases calcium influx. Here, we show that nicotinic acetylcholine receptor is also involved in the process of generating hyperpolarization in cultured chick myoblasts. Intracellular calcium level slightly increased by treatment of acetylcholine or nicotine. In addition, single-channel recordings revealed that acetylcholine increases the activity of K_{Ca} channels due to the calcium influx. Furthermore, acetylcholine hyperpolarized the membrane potential from –7mV to –25mV. This effect was completely blocked by charybdotoxin, a selective K_{Ca} channel blocker and by a calcium-free external solution. These results suggest that acetylcholine-induced calcium influx activates K_{Ca} channels and in turn hyperpolarizes membrane potential in chick myoblasts.

D112 **Cloning and Expression of Estrogen-regulated Genes in Mouse Uterus in Delayed Implantation process by Differential Display**

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In the mammals, the implantation of fertilized egg onto uterus is a critical step of further development and reproduction, however, little is known about genetic regulation of implantation process. Implantation consists of complicate and yet precise series of interaction between embryo and uterine epithelial cells, and this event is under control of ovarian steroids, such as estrogen and progesterone. In the present study, we employed the differential display RT-PCR (DD RT-PCR) technique to clone estrogen-regulated novel genes 24 hours after estrogen injection using delayed implantation model. Total RNA was extracted from whole uterine horns of vehicle- or estrogen-treated mouse. By DD RT-PCR, we found 120 putative estrogen-regulated genes. To verify the expression pattern, mRNA levels of 120 genes were simultaneously analyzed by reverse Northern blotting. Five of 120 genes were altered 24 hours after estrogen treatment, but the others showed no or little changes. Then, tissue specificity of these 5 genes was examined by Northern blot analysis: Clone #10 showed specific expression at the reproductive organs, and the others are expressed at a variety of tissues. Several clones including clone #2, #3, and #4 are new genes, while clone #8 contains viral LTR sequence and clone#10 is mouse oral tumor suppressor gene. Time course change was examined by Northern blot analysis. Clone #10 were down-regulated 6 hours after estrogen treatment and the other clones were down-regulated 24 hours after estrogen treatment. Overall, the present study revealed a set of genes was tissue-specifically and time-dependently regulated by estrogen at the time of implantation.