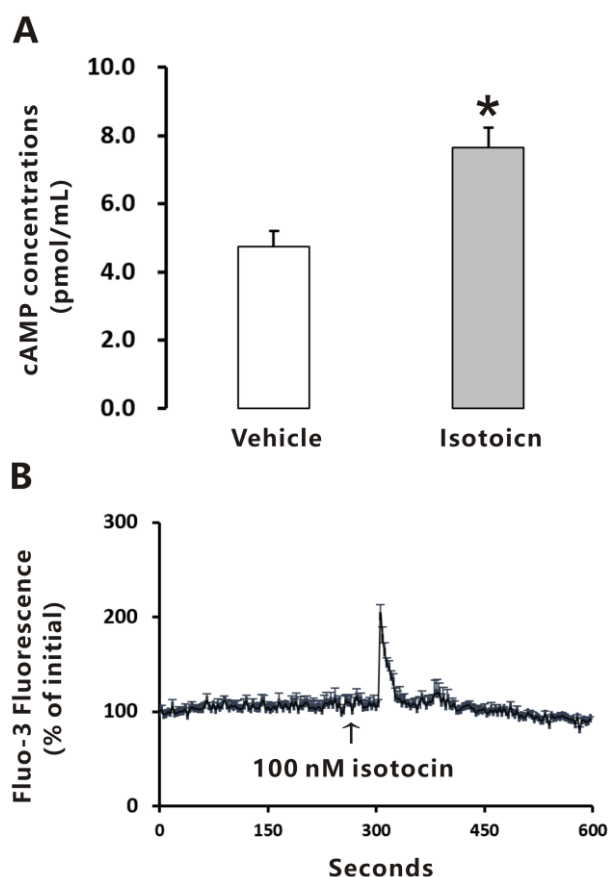


Supplemental Fig. 8



Supplemental Figure 8. Activation of cAMP and  $\text{Ca}^{2+}$  signaling pathways by isotocin in primary pituitary cells of female ricefield eels. A, effects of isotocin on cellular cAMP contents. The pituitary cells were pre-incubated for 12 hrs before being treated with isotocin (100 nM) for 12 hrs. After treatment, the amount of cAMP in the pituitary cells was quantified with a Monoclonal Anti-cAMP Antibody Based Direct cAMP ELISA Kit (catalog number 80203, NewEast Biosciences, Inc., PA, USA). Results are expressed as measured cAMP concentrations in pituitary cell homogenates. Bars represent means  $\pm$  SEM (n=4). \*P<0.05 vs the vehicle control. B, effects of isotocin on intracellular  $\text{Ca}^{2+}$  levels. The primary pituitary cells were plated onto cell culture dish, loaded with 5  $\mu\text{M}$  Fluo-3 AM. The fluorescence intensity in single cells was analyzed with a laser scanning confocal imaging system (TCS SP5; Leica Microsystems, Mannheim, Germany), and expressed as percentage of the initial Fluo-3 fluorescence.  $\text{Ca}^{2+}$  levels are presented as mean fluorescence intensities  $\pm$  SEM (n=3) of three independent experiments.