The Effects of uPA Inhibition on Cranial Neural Crest Migration

Objectives/Goals
To examine the effects of Urokinase-type Plasminogen Activator (uPA) inhibition on the normal migration of cranial neural crest cells in the early chick embryo.

Methods/Materials
After incubation to proper stages, chick embryos were cultured using either New Culture or Window Culture to allow access to the embryo while promoting normal growth. A 1mM solution of Amiloride was added either by dripping onto the embryo or injecting with a Picospritzer. After reincubation, embryos were fixed in 4% Paraformaldehyde and immunohistochemistry was performed for Hnk-1. Staining allowed for the visualization of neural crest cell locations. Each stage (4, 7, or 8) had between 50-70 embryos in each group. Percentages were employed for data analysis.

Results
The inhibition of uPA drastically reduced the number of embryos that showed some sort of neural crest migration (14% instead of 98% in controls). Stage 4 (18 hours incubation) embryos showed that uPA has a direct effect on the ability of neural tubes to close. Every single control embryo had normal closure, while amiloride-treated embryos only had 9% successful closure. A peculiar morphological effect of uPA inhibition is the bifid heart. Although not shown on the majority of embryos, a total of 12 experimental embryos showed the bifid heart characteristic.

Conclusions/Discussion
The inhibition of uPA does indeed halt cranial neural crest migration. Most results showed a clump of premigratory neural crest cells in the neural tube. Inhibition of uPA also prevented neural tube closure, the cause of Spina Bifida in humans. By understanding how neural crest is prevented from migrating and how neural tubes remain open, I can apply this towards developing methods for the promotion of neural crest migration in cells that have failed to do so.

Summary Statement
My project focuses on the effects uPA play on normal neural crest migration; inhibiting uPA allows for the determination of its typical role in early embryonic development.

Help Received
Lab funding and equipment used under the guidance of Dr. Mark A.J. Selleck at the USC Keck School of Medicine