CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Catecholamine Transport

Purpose
Study the kinetics of transport of various catecholamines across a biologically active membrane. Specifically, this report studies the in vitro transport of epinephrine(EPI), norepinephrine(NE), dopamine(DA), and isoproterenol into chromaffin ghosts.

Existing Methods
Utilize labeled catecholamines to study the in vitro uptake and release of amines in these secretory granules. The use of radiolabeled amines in this type of study has many drawbacks, which include, separation of granules and medium, leakage of the catecholamine from granules during isolation, exchange of labeled and unlabeled amines during the study, and the labor-intensive nature of kinetic measurements of amine transport.

Reference
Kinetic and Quantitative Measurements of Catecholamine Transport in Chromaffin Ghosts Using a Glassy Carbon Electrode, S. Hayflick, R.G. Johnson, S. E. Carty, and A. Scarpa, Anal. Biochem. 126 (1982) 58-66.

EC Equipment
CV-1A Voltammetry Controller. A glassy carbon voltammetry working electrode maintained at +0.5 V vs Ag/AgCl reference electrode. The output was monitored with a strip-chart recorder.

Notes
The LC-4B can be used if the transport of more dilute solutions than reported here is to be studied. The gain of the LC-4B is greater than that of the CV-1A. The same three electrode setup would be used.

Linearity
The response of the system was linear over the range 2 - 40 μM of amine in the incubation medium.

Experimental
Intact chromaffin granules were first isolated by differential and gradient centrifugation. The granules were lysed and washed free of endogenous catecholamines, then ghosts were formed by suspension of the membranes in buffered 0.185 M KCl. The experimental set up consisted of a thermostated chamber containing the stirred experimental medium and the chromaffin ghosts. The electrodes were immersed in the mixture, and the working electrode was maintained at +0.5 V vs. Ag/AgCl. Transport was initiated by the addition of the test amine or ATP. The results of one experiment are presented in Figure 1. Addition of EPI resulted in a rapid upward deflection as the EPI was oxidized at the surface of the working electrode. The subsequent time-dependent drop in the signal indicated that the EPI in the medium was being depleted (transported into the ghosts). Thus, it was not free in the solution and amenable to oxidation at the electrode. Since virtually all the EPI was transported into the ghosts, the accumulation must have been against a concentration gradient. Addition of NH_4^+

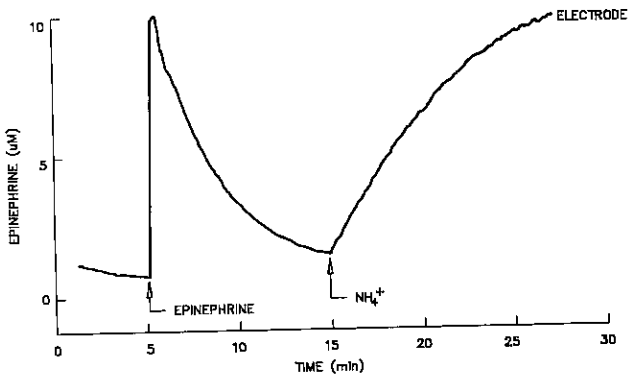

Figure 1. Uptake and release of EPI by chromaffin ghosts as measured by oxidation at a glassy carbon electrode in a stirred solution. Redrawn from above cited reference.

caused a complete release of the EPI from the ghosts, as indicated by the increase in signal after its addition. The EPI was then free in the medium and available for oxidation at the electrode surface. Decrease in the EPI concentration due to electrochemical oxidation was minimal, since the final signal is equivalent to the starting signal. HPLC analysis confirmed these results.

The advantages of the system outlined above for the study of catecholamine transport across membranes are discussed in the reference cited above.