Nicotinic Receptor-mediated Catecholamine Secretion from Individual Chromaffin Cells

CHEMICAL EVIDENCE FOR EXOCYTOSIS*

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Nicotinic receptor-mediated secretion of catecholamines from individual cultured bovine adrenal medullary chromaffin cells was measured and characterized with a voltammetric microelectrode placed adjacent to the cells. Nicotineduced secretion is associated with a large increase in chemical spikes that is temporally resolved into the apparent secretion of discrete packets of attomole quantities of easily oxidized molecules. These data are consistent with direct chemical measurement of single exocytotic events.

Chemical neurotransmission is thought to occur by the process of exocytosis where neurotransmitters, contained in synaptic vesicles, are extruded into the extracellular space following fusion of the vesicle and plasma membranes (1). Considerable evidence exists to support this hypothesis in the case of cholinergic neurons (2). Although acetylcholine secretion has been measured from the growth cones of single neurons with outside-out patches on micropipettes (3, 4), exocytotic events that do not have the time resolution to measure individual molecules. These data are consistent with direct chemical measurement of single exocytotic events.

To investigate catecholamine release from single cells, chromaffin cells were isolated and plated (10) at a density of 6 x 10⁵ cells/35-mm culture dish. Following at least 3 days of culture in Dulbecco's modified Eagle's medium and Ham's F-12 (1:1) supplemented with 10% fetal bovine serum, the culture medium was replaced with a balanced salt solution (11). Catecholamines were detected with beveled carbon fiber electrodes used in an amperometric mode or with fast-scan cyclic voltammetry (12). The electrode tip was positioned under an inverted microscope on a vibration-isolation table with a micromanipulator so that it was <5 μm from a single cell (Fig. 1). The cell radius is approximately 8 μm which is similar to the dimensions of the tip of the electrode. Chemical agents for stimulation were dissolved in balanced salt solution and introduced with a pulled-glass micropipette positioned approximately 20 μm from the cell (Fig. 1) with a pressure ejection system (Picospitzer, General Valve Corp., Fairfield, NJ).

In order to demonstrate the time course of the chemical stimulus, we ejected norepinephrine from the micropipette. Cyclic voltammetry of norepinephrine (Fig. 2A) is similar to that of dopamine and epinephrine, but is distinct from all other known vesicular components. Fig. 2C shows a typical temporal response measured in the cyclic voltammetry mode to the ejection of 20 μM norepinephrine in the absence of a cell. This time course simply indicates the concentration profile that will occur at the cell surface for any chemical introduced from the micropipette. Exposure of a cell to 100 μM nicotine results in the trace shown in Fig. 2D. The cyclic voltammogram ensures that the detected substances are catecholamines (Fig. 2B). Exposure of chromaffin cells to this dose of nicotine resulted in catecholamine secretion having a maximal concentration of 17.9 ± 1.4 μM (n = 15 cells). Similar amplitudes and cyclic voltammograms were observed with electrodes coated with a perfluorinated cation exchange polymer, an electrode that has even greater specificity for catecholamines (13).

Several investigators have shown that the nicotine-induced secretion of catecholamines from cultured adrenal chromaffin cells is sensitive to nicotinic receptor and Ca²⁺ channel blockers (5-7, 14-17). Hexamethonium and Cd²⁺ also decreased the electrochemical signal induced by nicotine from single chromaffin cells. However, the most unique feature of our results is the large chemical spikes superimposed on the secretion envelope. Cyclic voltammograms recorded during a single nicotine-induced spike show that a concentration packet of catecholamines causes the observed noise. In contrast, spikes are not observed with pressure ejection of nicotine in the absence of a cell or by ejection of a balanced salt solution from the micropipette onto an adjacent cell. Nicotine-induced spikes are still apparent at electrodes that are moved away from the cell by a few micrometers, although their amplitudes are reduced.

To improve temporal resolution, measurements were made in an amperometric mode at a fixed oxidizing potential. The response to 100 μM nicotine was qualitatively similar; however, the spikes became more clearly resolved with random amplitudes and rapid rise and decay times (Fig. 3A). The most rapid half-rise time observed for a single spike was 7 ms, the response time of the instrumentation. In some experi-
from single adrenal medullary chromaffin cells. Drawing is approximately to scale.

Fig. 1. Experimental arrangement for measuring secretion from single adrenal medullary chromaffin cells. Data were recorded with an EL-400 potentiostat (Ensman Instrumentation, Bloomington, IN) interfaced to an X-style personal computer, and a sodium-saturated calomel electrode (SSCE) was employed. Panels A and B are averaged background-subtracted voltammograms of the substances whose concentration changed during the measurement interval of panels C and D. Each time point in panels C and D is the integration current recorded from 0.5-0.6 V from individual voltammograms; bars to the right in panels C and D are the conversion of current to catecholamine concentration based on calibration curves constructed with standards. A, C, electrode response to 1-s 3-nl ejection of 20 μM norepinephrine applied at t = 0 with the ejection pipette 20 μm from the electrode. B, D, electrochemical response obtained with the electrode tip adjacent to a single cell; at t = 0, a 1-s ejection of nicotine (100 PM) was made to μm away from the cell.

Fig. 2. Cyclic voltammetric response (200 V s⁻¹, repeated at 100-ms intervals) of a carbon fiber electrode to norepinephrine ejection from an adjacent micropipette and to catecholamine secretion from a single chromaffin cell. Data were recorded with an EL-400 potentiostat (Ensman Instrumentation, Bloomington, IN) interfaced to an XT-style personal computer, and a sodium-saturated calomel electrode (SSCE) was employed. Panels A and B are averaged background-subtracted voltammograms of the substances whose concentration changed during the measurement interval of panels C and D. Each time point in panels C and D is the integrated current recorded from 0.5-0.6 V from individual voltammograms (hatched lines in panels A and B); bars to the right in panels C and D are the conversion of current to catecholamine concentration based on calibration curves constructed with standards. A, C, electrode response to 1-s 3-nl ejection of 20 μM norepinephrine applied at t = 0 with the ejection pipette 20 μm from the electrode. B, D, electrochemical response obtained with the electrode tip adjacent to a single cell; at t = 0, a 1-s ejection of nicotine (100 PM) was made 20 μm away from the cell.

Fig. 3. Current measured from two electrodes (Eapp = 0.65 V) placed on opposite sides of a single cell. Signals measured following ejection of 100 μM nicotine at t = 0 (A, B). C, D, expanded time and current scale of the boxed area indicated in panels A and B. Averages of 200 ms in increments (n = 9) two uncoated electrodes were placed on opposite sides of an individual cell to simultaneously probe the spatial dependence of the spikes. Spikes were observed at either side of the cell (Fig. 3, A and B) but appeared at different variable times as seen in the expanded scale (Fig. 3, C and D).

REFERENCES