ACTIVATION AND INACTIVATION OF TETANUS TOXIN
IN CHROMAFFIN CELLS

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INTRODUCTION

Tetanus toxin is produced by Clostridium tetani as a single chain, almost non-toxic, protein with a molecular weight of approximately 150,000 representing 1315 amino acids. Bacterial proteases cleave the molecule between positions A 457 and S 458 (extracellular activation), yielding a heavy chain (MW 100,000) and a light chain (MW 50,000) tetanus toxin (HC-TeTx, LC-TeTx). Both chains remain connected to each other by a disulphur bond between positions C 439 and C 467 (Dichain-TeTx). The cleavage or nicking dramatically increases the biological activity². HC-TeTx is involved in binding DC-TeTx to gangliosides lodged in the plasma membrane, which is a prerequisite for incorporation into the cytosol³. Whether DC-TeTx diffuses into the cells through pores formed by H-CTeTx⁴ or is taken up by receptor-mediated endocytosis is not yet clear. Within the cells, the disulphur bond between the two chains is reduced to separate LC-TeTx from DC-TeTx (intracellular activation). Toxicity is carried exclusively by the reduced form of LC-TeTx⁵. The intracellular target of the toxin is present in several types of neuronal and endocrinial cells, such as cholinergic, noradrenergic, glycinergic, GABAergic, and other neurons⁶ and also chromaffin cells⁷. Therefore they all respond to the toxin. However, since chromaffin cells lack gangliosides in their plasma membrane⁸ they cannot take up the toxin unless special manipulations are performed. Nevertheless, chromaffin cells have firmly been established as useful tools for studying the interference of TeTx with exocytosis, because they represent a homogenous population and offer an excellent access to structures controlling hormonal release. There are several ways to introduce TeTx into their cytosol. It can diffuse through plasma membrane pores induced by cytolysins⁴, and it can be injected⁷, or taken up by exogenous gangliosides incorporated into the plasma membrane⁹. Cytolysine-permeabilised chromaffin cells, in contrast to ganglioside-enriched or micropipette-porated cells, respond only to LC-TeTx or to chemically reduced DC-TeTx⁵, indicating that, during their short survival time, these cells are not able to cleave the sulphur bonds.
Electroporation provides definite advantages over permeabilisation by means of cytolysins, because those cells that survive the actual procedure, continue to live for days. Using this technique we were able to show that chromaffin cells have the enzymatic tools to activate DC-TeTx and that the toxin’s effect on exocytosis is not irreversible. However, neutralisation or degradation of the toxin is a necessity for the recovery of exocytosis.

RESULTS AND DISCUSSION

Exocytosis from Permeabilised and Intact Chromaffin Cells

Permeabilisation had a damaging effect on chromaffin cells. Cytolysin formed permanent pores in the plasma membrane causing a continuous decrease of exocytotic activity (Fig. 1). Electroporation, on the other hand, produced transient pores with a diameter of up to 1μm in those areas of the plasma membrane that faced the electrodes of the poration cuvette during current flow (Fig. 2). Proteins, like tetanus toxin or antibodies, diffuse through these openings and remain trapped inside the cells when the pores are closed by membrane fusion. The closure allows a complete recovery from the damage as judged from the restoration of exocytosis which increased to the level found in control cells (Fig. 1).

![Figure 1. Exocytosis in permeabilised chromaffin cells. Chromaffin cells were loaded with 3H-noradrenaline for two hours. After washing they were permeabilised with 20μM digitonin (closed circles) for 10 min or with 200U/ml of staphylococcal α-toxin (open triangles) for 30 min. Then they were further incubated in plain medium as indicated (abscissa). The release of 3H-noradrenaline was stimulated with 10μM Ca++ 11. The release was compared with the release from a twin sample stimulated immediately after permeabilisation. Other sets of cells were electroporated or left intact. After different periods of maintenance (abscissa) in monolayer culture 3H-noradrenaline release was stimulated with carbachol. The release from electroporated cells was compared to the release from the non-permeabilised control cells (open circle). The normalized release of 1.0 means that the release from permeabilised cells equals that from intact cells.](image-url)