Chromaffin granules as target for the antihypertensive drug hydralazine.

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Several drugs are known to modify catecholamine release from adrenal chromaffin cells with regards to the kinetics and quantal size[1 3]. Here we asked whether the beneficial effects of hydralazine (HYD), a drug used in the treatment of certain forms of drug-resistant hypertension, might be explained by its interference with catecholamine storage, or exocytosis. Our results demonstrate that, even at nanomolar concentrations, HYD accumulates rapidly into secretory granules, thereby causing dramatic changes in the kinetics and the quantal size of individual release events.

RESULTS AND DISCUSSION

Carbon fiber amperometry is a powerful tool for the study exocytosis. It allows measurements of release kinetics at the level of individual exocytotic events and of the apparent quantal size[4]. Using this technique we found that acute application of HYD (10 nM) slowed the rate of transmitter release (Imax from 22.9±2.3 to 13.9±1.9 pA), which was accompanied by a 35 % decrease in quantal size (Q) (Figure 1a). Notably, spike firing frequency was not significantly altered by the drug.

HYD is a weakly fluorescent compound that emits light at 405 nm when is excited at 483 nm. Confocal microscopy of chromaffin cells that were preincubated with HYD revealed that the drug rapidly accumulates into vesicular structures (not shown). The pattern of distribution within the cell was similar to that of the dye acridine orange (AO), which is known to accumulate in secretory granules. We studied the time course of HYD accumulation by standard epi-fluorescence microscopy (Figure 1B). In control conditions, uptake of HYD (10mM) into chromaffin granules occurred with a half-time of 300 s. Interfering with granular acidification by preincubation with 100 nM bafilomycin A_1 prevented accumulation of the drug. This suggests that HYD is mostly compartmentalized by protonation within chromaffin granules, although accumulation in other acidic organelles may occur as well.

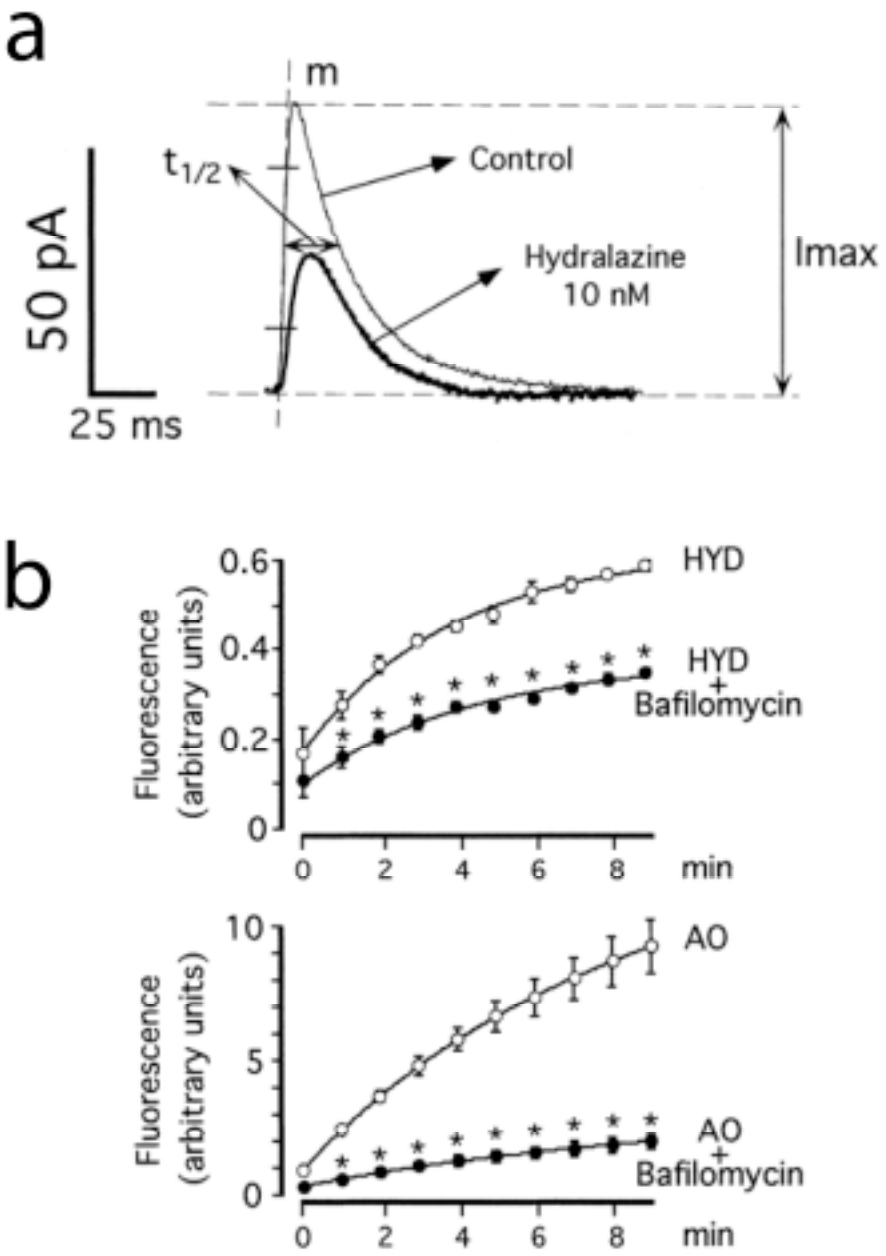

Figure 1. a) Representative spike traces were plotted using the kinetic parameters from averaged values. Hydralazine was applied as a brief 5-second

pulse together with $BaCl_2$ (5 mM). Vertical and horizontal bars are the calibration for the oxidation current and time. **b)** Time course of hydralazine and acridine orange accumulation in single bovine adrenal chromaffin cells. Hydralazine was applied for 10 seconds by pressure at 10 μM. Data also show the effects of incubation with 100 nM bafilomycin. Acridine orange was applied for 10 seconds at 10 nM in the presence or in the absence of 10 nM bafilomycin.

The decreased quantal size in presence of HYD, combined with its granular accumulation suggests the drug may act by displacing the catecholamines to the cytosol. To address this possibility we measured cytosolic catecholamine contents by permeabilizing cells with a 10-second pulse of digitonin (20 μM) in the absence of extracellular Ca^{2+} [5]. Under these conditions digitonin application did not evoke secretory spikes. Any current evoked at the carbon fiber electrode should therefore reflect release of cytosolic catecholamine. When digitonin was applied on a HYD-treated cell, this elevation was significantly increased (2.5 times). This effect is similar to that recently described in chromaffin cells for other weak bases like tyramine and amphetamine[6,7]. In summary, our data suggest that HYD acts by displacing catecholamines from the granule, thus causing a reduced quantal size slowed kinetics of transmitter release. On the systemic level, similar changes in sympathetic neurons would be expected to cause a significant drop in catecholamine release and this could explain many of the therapeutic and side effects of this drug.

A long version of this work you can find in reference 8.

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