Amperometric secretory spikes in mouse adrenal slices.

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Most studies to analyse the kinetic of the exocytotic catecholamine release responses have been performed in primary cultures of chromaffin cells dissociated from adrenal medullary tissues, mostly from bovine and rats, using non-oxygenated Krebs-HEPES (KH) solutions lacking bicarbonate and phosphate anions. In this work, we have used single chromaffin cells in acute fresh mouse adrenal slices, bathed in Krebs-bicarbonate solutions equilibrated with 95% O_2/5% CO_2 (BBS), a preparation closer to the physiological situation.

With the use of carbon fiber microelectrodes, we have compared the kinetic properties of the amperometric exocytotic spikes obtained in cells in slices, with those obtained in mouse chromaffin cells in culture (2-4 days), as well as the kinetic of the spikes obtained in slices bathed in Krebs-HEPES solution, instead of BBS. To get the amperometric secretory spikes, 5 s pulses of solutions of BBS or KH containing acetylcholine (ACh) or high K^+ were applied.

Here, we show that the kinetic of the amperometric secretory spikes in culture cells differ not very much from those obtained from slices. Besides, we also demonstrate that the components of both types of the bath solution used are not of critical importance to modulate the kinetic of the exocytosis.

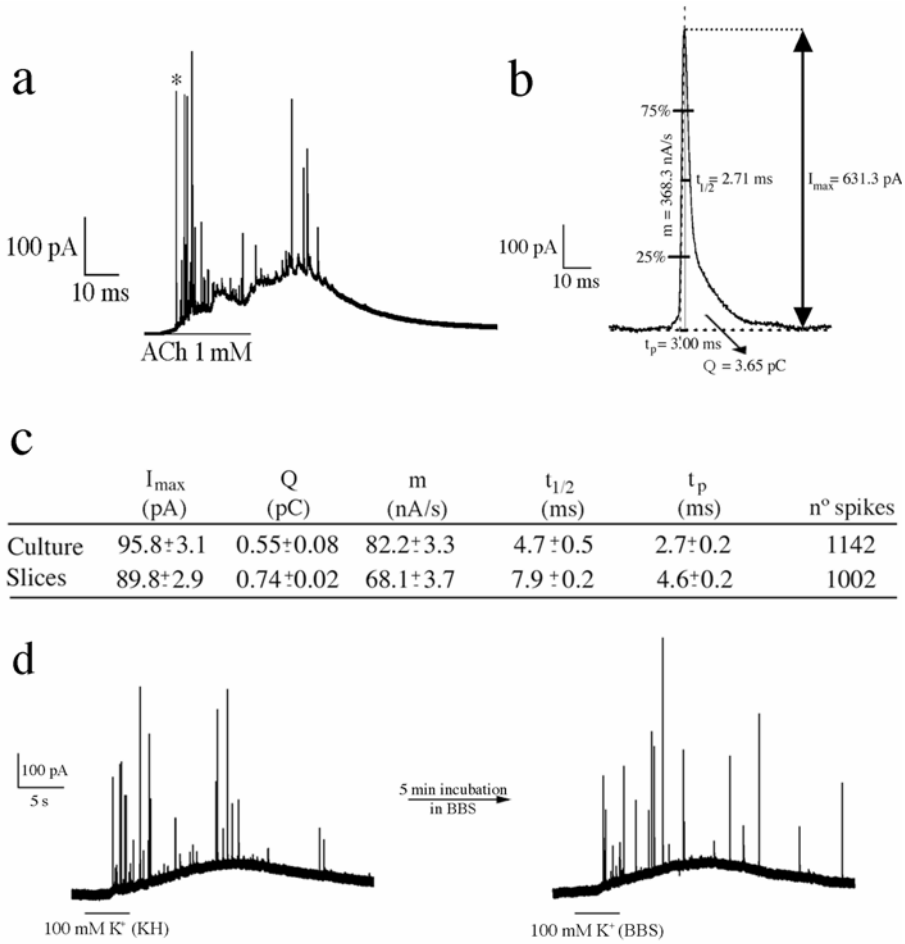
RESULTS AND DISCUSSION

Most of the experiments in the literature have been mainly performed in isolated and cultured cells, using prolonged (minutes) depolarizing pulses of high K^+ or action potentials, probably due to technical complexity of using slices of tissue, bathed with oxygenated bicarbonate-based solutions.

To characterize the amperometric exocytotic signals in chromaffin cells of mouse adrenal slices, exogenous application of 1 mM ACh or 100 mM K^+ in BBS-based solutions was first tried (figure 1a). A burst of amperometric spikes was recorded after 5 s of stimulation. Typical single fast amperometric event evoked by ACh, with a detailed analysis of its kinetic parameters, is shown in figure 1b. The following kinetic parameters were measured: I_{max} (peak amplitude); Q (charge); m (ascending slope, calculated from the linear part of the trace located between 25% and 75% of the I_{max}); $t_{1/2}$ (half-

width or duration of the amperometric signal at 50% of its peak amplitude); and t_p (time-to-peak, determined between the point at which the back-extrapolation of the slope line crossed the base line at the point of I_{max}). The average values of the spike kinetic parameters obtained (808 spikes when the stimulus was ACh, and 756 spikes when the stimulus was K^+, from 7 cells) are: 114±4.10 pA; 1.00±0.03 pC; 73.5±3.47 nA/s; 7.9±0.20 ms; and 4.6±0.17 ms for ACh and 99.1±3.45 pA; 0.82±0.03 pC; 68.9±3.92 nA/s; 7.0±0.16 ms; and 4.4±0.33 ms for K^+.

These data exhibit drastic and important differences with those published by Herrero and coworkers[1] in cultured mouse chromaffin cells. These authors perfused 1 mM ACh in HEPES-based solutions for 5 s, obtaining amperometric spikes with the following parameters: I_{max}=22.8±1.8 pA; Q=0.48±0.02 pC; m=5.2±0.6 nA/s; $t_{1/2}$=19.9±6.1 ms; t_p=22.5±1.5 ms (n=250 spikes). To be able to elucidate if differences were due to the physiological preparation (culture vs slices) or to the perfusion solution (BBS- vs HEPES-based solutions), additional experiments in chromaffin cells in culture, using BBS as the perfusion solution, were performed (figure 1c). Statistical analysis, carried out by the non-parametric Mann-Whitney U test, revealed that spikes from culture cells have shorter times of release ($t_{1/2}$: 4.7±0.5 vs 7.9±0.2 ms; t_p: 2.7±0.2 vs 4.6±0.2 ms). We think these are very surprising results since cells in slices should show a higher quality characteristics than cells in culture. We believe that this kind of stimulus application, by means of a multibarrelled glass pipette positioned closed to the cell under study, also stimulate a great number of neighbouring cells. Catecholamines secreted by them could as well be detected for the carbon fiber electrode, which would reach it with a very different kinetic. Probably, this is the reason why we have found extremely fast and large single events accompanied by other of slower and smaller amplitude in the same recording in slices. So, we guess that average kinetic parameters of pooled results shown in figure 1c are distorted by those amperometric events coming from neighbouring cells to the one being recorded.



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	I_{max} (pA)	Q (pC)	m (nA/s)	$t_{1/2}$ (ms)	t_p (ms)	nº spikes
Culture	95.8±3.1	0.55±0.08	82.2±3.3	4.7±0.5	2.7±0.2	1142
Slices	89.8±2.9	0.74±0.02	68.1±3.7	7.9±0.2	4.6±0.2	1002

Figure 1. Amperometric exocytotic events obtained in different experimental conditions, recorded from a single cell of mouse adrenal medulla. a) Original recording obtained with 5 s stimulus of 1 mM ACh. **b)** The amperometric spike marked with an asterisk in a) is represented in an expanded time scale with its kinetic parameters indicated. **c)** Detailed analysis from individual spikes in cells isolated and maintained in culture (n=20, n=1142 spikes), and in cells in slices (n=14, n=1002 spikes), following the protocol in a) using 100 mM K^+ as a stimulus. **d)** Typical amperometric recordings of a cell in slice of tissue superfused with KH and BBS, following the protocol in a).

Afterwards, we explored the effect of the buffer solutions (BBS vs KH) on catecholamine secretion. To elucidate this point, we performed experiments stimulating the slice while bathing in BBS or

in KH, alternatively. After several stimulations in one of the buffers, the slice was then superfused during 5 min with the other, before applying a new stimulation (figure 1d). This sequence was repeated one more time (not shown) in order to be sure that the level of secretion hadn't decreased during the experiment. Then, using different slices, we performed the same protocol but changing the buffers order, and similar results were found. Analysis of amperometric spikes from mouse adrenal slices after superfusion with KH and BBS was, respectively: I_{max}: 98.2±2.88 vs 96.6±3.35 pA; Q: 0.72±0.02 vs 0.67±0.02 pC; m: 77.9±5.76 vs 76.0±4.15 nA/s; $t_{1/2}$: 6.3±0.11 vs 6.0±0.14 ms; t_p: 3.85±0.02 vs 3.80±0.01 ms. Data are pooled from 944 (KH) and 616 (BBS) individual secretory spikes, from 10 and 8 cells, respectively.

These results imply the following conclusions between single secretory amperometric events between mouse adrenal chromaffin cells in situ (fresh slices) and cultured cells: (1) ACh and high K^+ produced similar secretory responses; (2) no differences were observed between cells in culture and in slices, when the following kinetic parameters are compared: I_{max}, Q and m. However, amperometric spikes from cells in culture exhibit smaller $t_{1/2}$ and t_p than those in slices, probably due to the contamination with secretory vesicles coming from neighbour cells; (3) no differences were seen in the kinetic parameters of spikes when cultured cells or slices were superfused with oxygenated BBS or with non-oxygenated KH solutions and (4) amperometric secretory events described here are much faster than those previously reported[1]. This suggests that mouse chromaffin cells undergo exocytosis following a fast kinetic pattern, which approaches that of neurons[2].

In conclusion, we have recorded extremely fast spikes in chromaffin cells in situ ($t_{1/2}$=1.66±0.05 ms, m=234.0±4.1 nA/s), much faster than that previously reported using ACh as a stimulus in cultured mouse chromaffin cells ($t_{1/2}$=19.9±6.1 ms, m=5.2±0.6 nA/s)[1]. We do not know the exact reasons for it, but it is plausible that the different experimental conditions used such as slice versus cultured cells, or even the different sampling rate, 14.5 kHz versus 1kHz, explain these different results.

REFERENCES

1. Herrero, C.J. et al., *Modulatory mechanism of the endogenous peptide catestatin on neuronal nicotinic acetylcholine receptors and exocytosis.* J Neurosci, 2002. **22**:377-388.
2. Bruns, D. and Jahn, R, *Real-time measurement of transmitter release from single synaptic vesicles.* Nature, 1995. **377**:62-65.