Quantal release of catecholamines. A new target for β-adrenergic antagonists.

Mónica S. Montesinos, Marcial Camacho, Jésica Díaz and Ricardo Borges

Unidad de Farmacología. Facultad de Medicina. Universidad de La Laguna. Tenerife, Spain.

Correspondence: Dr. Ricardo Borges, Unidad de Farmacología, Facultad de Medicina, Universidad de La Laguna, E-38071-La Laguna,Tenerife. Spain
Phone: 34-922-319346; **Fax:** 34-922-655995; **Email:** rborges@ull.es

Cell Biology of the Chromaffin Cell
R. Borges & L. Gandía Eds.
Instituto Teófilo Hernando, Spain, 2004

The use of β-adrenergic blockers (β-B) in the management of hypertension has been lasted for decades. However, its precise mechanisms of action remain obscure because their putative targets (cardiac output, renin secretion or CNS) are not common to all β-B and because the onset of hypotensive effect is delayed several days upon the initiation of therapy.

One possible alternative target is the interaction with the quantal release of catecholamines that sustain the arterial tone. It is known that β-B progressively reduce the arterial tone, which is difficult to understand because β_2 receptor activation relaxes muscular arterioles.

Our hypothesis points towards to a β-B accumulation into secretory vesicles thus displacing catecholamines and the co-release of these drugs together with their agonist thus reducing its action on the postsynaptic cell. We have use amperometry on single chromaffin cell to measure the characteristics of the quantal release of catecholamines and a flow injection system to quantify the release of catecholamines and β-B from stimulated cells.

RESULTS AND DISCUSSION

In order to test the hypothesis of an intravesicular accumulation of the drug in chromaffin granules and its possible secretion by exocytosis, we take the advantage of the fluorescent properties of both β-B. Particularly, labetalol exhibits fluorescence within a light spectrum of wavelengths slightly larger ($\lambda_{335/420}$ nm) than propranolol ($\lambda_{305/354}$ nm) being more suitable for recording. Figure 1a shows the effect of repetitive pulses of 100 µM of the nicotinic agonist dimethyl-phenyl-piperazinium applied with a rotary valve with a 100 µl loop. Cells (2 million) are packaged in a perfusion chamber of a very low dead volume and perfused at 2 ml/min with a HPLC pump [6]. The effluent is passed successively through a fluorimetric detector and an amperometric detector to detect labetalol and catecholamines. Control and cells acutely incubated with labetalol release catecholamines as detected by amperometry but only very small signals come from the fluorescence detector. The origin of these signals could be due to the release of weakly fluorescent compounds released by vesicles like catecholamines. However, cells chronically incubated with labetalol

release the drug together with catecholamines thus increasing the ratio fluorescence/amperometry (figure 1b).

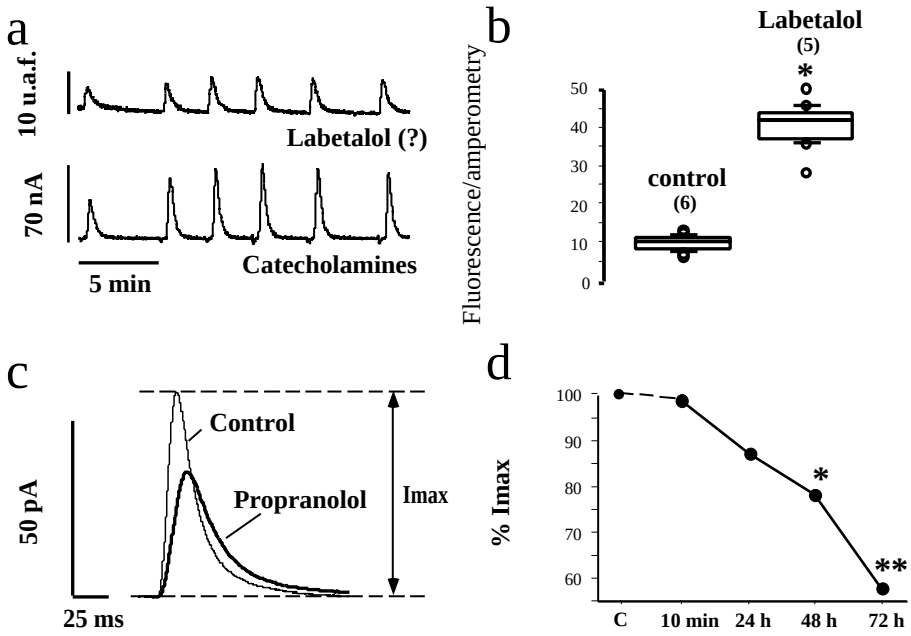

Figure 1. Beta blockers interact with the storage and release of catecholamines. a) Labetalol is released from chromaffin cells stimulated with pulses of DMPP 100 µM. Upper trace show the fluorescent signals whereas the lower show the amperometrical recording from chromaffin cells incubated 30 h with 10 µM of labetalol. **b)** Average from the ratios between fluorescent and amperometry (median ± percentile) from untreated cells (control) and treated (Labetalol). **c)** Representative spikes constructed with the average of kinetics parameters obtained from cells treated with 1 µM propranolol for 72 h. **d)** Normalized data showing the effect of incubation time with propranolol 1 µM on the Imax of secretory spikes. **p*<0.05, ***p*<0.01 student *t* test.

If β-B are entering into chromaffin granules it is likely that they compete with catecholamines interfering their storage. Amperometrical recordings do not exhibit any difference between control and cells that receive acute incubation with 0.1-10 µM of labetalol or propranolol. However, both compounds reduce slow down the exocytosis process and reduce the quantal size when are incubated for

1-3 days (figure 1c). These effects are evident even with low β-B concentrations (100 nM) and are more pronounced with propranolol than with labetalol probably due to the higher liposolubility of the former. The kinetics of exocytosis is more sensitive to β-B treatment than the quantal size. Both are time and concentration dependent (figure 1d).

Many β-Bs are weak bases that progressively accumulate into acidic compartments like chromaffin granules (pH 5.5)[5]. This process seems to be relatively slow when compared with other drugs like amphetamine[2-3] or hydralazine[9], which have rapid uptake. However, this slow time course is compatible with that observed in the clinical practice where several days are required to promote relevant reduction on blood pressure. To our knowledge this is the first time that these presynaptic mechanisms are proposed to explain the action of β-B on hypertension.

REFERENCES

1. Kumakura, K., et al., *Real-time monitoring of the secretory function of cultured adrenal chromaffin cells*. J Neurochem, 1986. **46:**1851-1858.
2. Boksa, P. *Studies on the uptake and release of propranolol and the effects of propranolol on catecholamines in cultures of bovine adrenal chromaffin cells.* Biochem Pharmacol, 1986. **35:**805-815.
3. Sulzer, D. and E.N. Pothos. *Regulation of quantal size by presynaptic mechanisms*. Rev Neurosci, 2000. **11:**159-212.
4. Machado, J.D., et al., *Hydralazine reduces the quantal size of secretory events by displacement of catecholamines from adrenomedullary chromaffin secretory vesicles*. Circ Res, 2002. **91:**830-836.