Intracellular distribution of serotonin and propranolol in rat aorta cells

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Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an important biomolecule that functions as both a neurotransmitter and vasoconstrictor[1,2]. It has recently been proposed that the smooth muscle cells of the aorta wall contain a complete serotonergic system, regulating the concentrations of 5-HT in arterial blood[3]. According to this proposition, the smooth muscle cell membranes contain a serotonin transporter (SERT) through which serotonin is actively accumulated. The uptake of serotonin into rat aortic smooth muscle cells and peripheral arteries was confirmed by Ni *et al.*[4] using immunocytochemistry. The serotonin transporter (SERT) of the smooth muscle cell plasma membrane may also be involved in the mediated transport of the beta-blocker drug propranolol[5] in addition to the usual transport by alpha-2 adrenoceptors[6]. Propranolol has been used for the treatment of cardiovascular diseases since it reduces arterial blood pressure by diminishing peripheral vascular resistance. However, the mechanisms for this vasodilatory effect have not been thoroughly investigated[7].

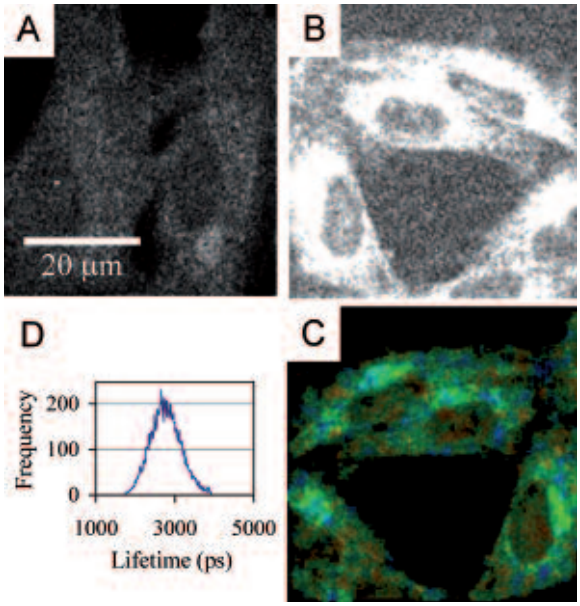

Figure 1. RAOSM cells at 37° C before (A) and 64 minutes after (B) addition of serotonin (250 µmol dm^{-3}) to the medium. C shows the fluorescence lifetime map from B, and the distribution of lifetimes is shown in D.

Our recent work at the LSF has established 2-photon excited fluorescence lifetime imaging of serotonin as a useful method to study the uptake and quantify the distribution of serotonin in living mammalian cells[8]. In these experiments the normal ultraviolet fluorescence of serotonin at 340 nm is excited in a two photon process by a Ti:sapphire laser and optical parametric oscillator (OPO) producing 180 fs pulses at 630 nm. Scanning of the focussed laser beam and fluorescence measurements at each pixel using time-correlated single photon counting allows pseudo-confocal fluorescence lifetime imaging with sub-micrometer resolution. The ability to measure both fluorescence intensities and lifetimes allows intracellular fluorophore concentrations to be determined after correction for dynamic quenching of fluorescence. In the experiments reported here, this method has been used to image both serotonin and propranolol using these wavelengths in live cultured rat aortic smooth muscle (RAOSM) cells.

Results and discussion

a) Serotonin imaging in RAOSM cells

Figure 1 shows images obtained of adherent RAOSM cells maintained at 37°C before (Fig 1A) and after (Fig 1B) addition of serotonin (250 µM) to the growth medium. The lifetime image (Fig 1E) and plot of lifetimes distribution (Fig 1D) are also shown. As in our previous experiments[8,9], the lifetime image shows values peaking at 2.9 ns and somewhat lower than the fluorescence lifetime for serotonin in aqueous pH 7 buffer (3.8 ns). After calibration and adjustment for lifetime quenching, the peak brightness in Figure 1B is equivalent to a maximal intracellular serotonin concentration of approximately 1.5 mmol dm^{-3}, indicating a six-fold increase in concentration over that of the added external serotonin. It is clear from the value of the Stern-Volmer constant for self quenching of serotonin fluorescence ($k_q = 2.5 \times 10^9$ dm^3 mol^{-1} s^{-1}) that at this intracellular concentration, the reduction in lifetime must be due to environment effects rather than through self-quenching. Serotonin fluorescence is distributed in a relatively diffuse manner within the cell cytoplasm and excluded from the cell nucleus. Similar images (not shown) were obtained when RAOSM cells were allowed to accumulate serotonin at 20° C.

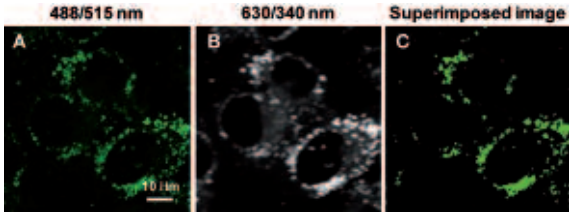

Figure 2. Images obtained after incubation of RAOSM cells at 20° C after incubation with dl-propranolol (100 µmol dm^{-3}). A:- Image obtained with further addition of LysoTracker green DND (488 nm excitation and 511 nm emission); B:- propranolol fluorescence at 340 nm excited at 630 nm; and C) superimposition of images A and B.

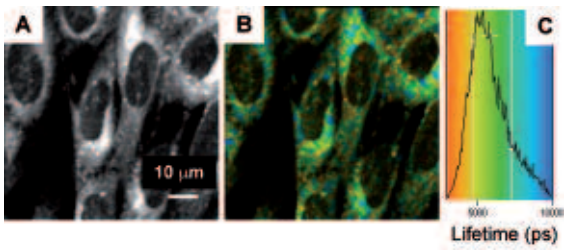

Figure 3. Fluorescence intensity (A) and lifetime images (B) of propranolol fluorescence (340 nm emission and 630 nm excitation) from RAOSM cells after brief incubation with propranolol (150 µmol dm^{-3}) at 37° C. The distribution of fluorescence lifetimes in B is illustrated in C.

b) Propranolol imaging in RAOSM cells

As previously shown [10], propranolol has excitation and fluorescence spectra quite similar to those of serotonin and so may be imaged under similar conditions. However fluorescence from the two compounds may be readily distinguished since the propranolol fluorescence lifetime (10 ns) is considerably longer than that of serotonin. Figure 2B shows the images obtained with 630 nm excitation of propranolol fluorescence at 340 nm after a brief incubation with dl-propranolol (150 µmol dm^{-3}) with RAOSM cells at 20° C. Under these conditions the propranolol fluorescence is highly localised within intracellular structures that are identified as lysosomes by co-localisation studies using Lysotracker green DND-26 fluorescence [11] and the confocal imaging accessory to the multiphoton microscope system. Figure 2A shows the LysoTracker image and Figure 2C the overlaid image of LysoTracker and propranolol fluorescence, confirming the co-localisation. In contrast incubation of RAOSM cells with propranolol at 37° C leads to the images shown in Figure 3. Although some localisation of propranolol fluorescence is observed at the higher temperature, there is also a more general diffuse distribution in the cell cytoplasm and exclusion form the nucleus. These images appear much more similar to those observed after serotonin uptake, although the peak concentrations of propranolol in the cytoplasm (between 10 and 25 mmol dm^{-3}) are an order of magnitude higher than those determined for serotonin. The distribution of fluorescence lifetimes observed from intracellular propranolol peaks at about 6 ns, shows a quenching of lifetime from the value of 10 ns in dilute solution that is largely consistent with the intracellular propranolol concentrations and the self-quenching rate constant previously obtained [10].

The results obtained for serotonin uptake in RAOSM cells may be compared with those previously present for rat leukemic mast RBL-2H3 mast cells. The mast cells contain specialised storage granules for serotonin in which the serotonin concentration may be up to ca. 50 mmol dm^{-3} and in which the fluorescence lifetime reduced to < 2 ns by self quenching. The present results appear to show that RAOSM cells lack such granules for serotonin storage and instead the accumulated serotonin is distributed throughout most of the cell cytoplasm. The observation that rat aortic smooth muscle cells are able to store both 5-HT and propranolol may be of clinical importance due to the influence that these two compounds exert at the cardiac level. Furthermore, peripheral arteries might play a role in the clearance of plasma 5-HT or propranolol through uptake and metabolism.

Acknowledgements

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