

**GABA SYNTHESIS AND RELEASE IN THE SINGLE-CELLED ORGANISM
*PARAMECIUM***

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The aim of this work is to verify the mechanism of gamma-aminobutyric acid (GABA) synthesis and release in the ciliated protozoon *Paramecium*. The presence of GABA_B R1 and R2 receptor subunits in *P. primaurelia* cells has been demonstrated by Western blotting and confocal scanning microscopy. Immunofluorescence was detected on the cell surface and throughout the cytoplasm. We have found that GABA_B receptors modulate swimming behavior inhibiting L-type calcium channels via G-proteins [1]. This observation suggests a protective action of GABA related to its ability to mediate an inhibition of calcium influx. According to this picture we have essayed the capability of *Paramecium* to synthesize and release GABA in the environment. By HPLC we have observed that the transmitter is spontaneously released in the environment but its amount significantly increases in the medium collected after a KCl (40 mM) -induced membrane depolarisation. By immunofluorescence and confocal microscopy we have evidenced the presence of glutamic acid decarboxylase (GAD), the enzyme responsible for the conversion of glutamate to GABA, of the vesicular GABA transporter (v-GAT) and of the proteins involved in transmitter release. The release implicates assembly of the v-SNARE vesicle-associated membrane protein (VAMP or synaptobrevin) and the t-SNARE syntaxin and synaptosomal-associated protein of 25 kDa (SNAP-25), which provide a link between docked vesicles and the plasma membrane. Therefore, K⁺-evoked membrane depolarisation causes in *Paramecium* the elevation of cytosolic calcium and the neurotransmitter release through a vesicular mechanism similar to that found in mammalian cells [2].

[1] P. Ramoino; F. Beltrame; A. Diaspro; M. Fato; L. Raiteri; S. Stigliani and C. Usai, "Swimming behavior regulation by GABA_B receptors in *Paramecium*", *Exp. Cell Res.*, **291**, 398-405 (2003).

[2] T. Sollner; M.K. Bennett; S.W. Whiteheart; R.H. Scheller and J.E. Rothman, "A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion", *Cell*, **75**, 409-18 (1993).