SYNAPTIC VESICLE PROTEIN SV2B, BUT NOT SV2A, IS PREDOMINANTLY EXPRESSED AND ASSOCIATED WITH MICROVESICLES IN RAT PINEALOCYTES

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We have shown that rat pinealocytes are glutaminergic endocrine cells, and secrete L-glutamate through exocytosis (reviewed in 1). The secreted L-glutamate binds to class II metabotropic glutamate receptor, and inhibits N-acetyltransferase activity through an inhibitory cAMP cascade (2). It is quite likely that pinealocytes use L-glutamate as a negative regulator for melatonin synthesis through paracrine- or autocrine-like intercellular signal transduction.

Microvesicles (MVs), endocrine counterparts of neuronal synaptic vesicles, are present in mammalian pinealocyte (3–5) and are responsible for L-glutamate exocytosis (6). Studies on the structure and function of the pineal MVs are important to understand the molecular events on the mechanism of the glutamate signal output. Our recent studies indicate that pineal MVs contain synaptophysin, synaptotagmin, VAMP2, and N-ethylmaleimide-sensitive fusion protein (NSF), but devoid of synapsins (Figure 1) (1). Furthermore, the MVs also possess vacuolar H+-ATPase (V-ATPase) as a main constituent (approximately 10% of total protein), and vesicular glutamate transporter. The V-ATPase and vesicular glutamate transporter are energetically coupled: L-glutamate transport is driven by membrane potential (inside positive) established by

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V-ATPase (Figure 1) (3,4). L-Glutamate inside MVs is exocytosed Ca^{2+}-dependently and appears in extracellular space (6). The exocytosis seem to occur with similar mechanism to neuronal synaptic vesicles and endocrine secretory granules. However, the rate of MVs exocytosis is about 1/10 of known regulated exocytosis (6,7), and detailed studies on the mechanism of exocytosis will be important to reveal the mechanistic similarity and difference of the known exocytosis.

To characterize the molecular organization of microvesicles in more detail, we investigated in this study the expression and localization of synaptic vesicle protein 2 (SV2), in rat pinealocytes. SV2, originally identified in cholinergic vesicles from the electric fish, Discopyge ommanta (8), is a glycosylated synaptic vesicle membrane protein comprising 12 transmembrane regions (9–11). Two isoforms, abbreviated as SV2A and SV2B, have been identified in mammals: the former consists of 742 amino acids and the latter of 683 amino acids, with 65% sequence homology (12,13).

Reverse transcription polymerase chain reaction (RT-PCR) analysis indicated that transcripts specific for two isoforms, SV2A, a ubiquitous form present in neuronal and endocrine cells, and SV2B, a neuron-specific form, are amplified in pineal RNAs. Northern blotting with specific transcripts indicated that the mRNA for SV2B is predominantly expressed, whereas that for SV2A is below the detection limit. Site-specific antibodies against SV2B recognized a single 72 kDa polypeptide in the pineal membrane fraction, whereas anti-SV2A antibodies did not recognize any polypeptides. Immunohistochemical analysis of cultured cells indicated that SV2B is expressed in pinealocytes but not in other types of cells. SV2B is present in somata and is especially rich in processes, which are filled with microvesicles. SV2B is co-localized with synaptophysin and synaptotagmin, markers for microvesicles. Immunoelectron-microscopy indicated that SV2B is associated with microvesicles. These results indicated that SV2B, a neuronal specific form, but not SV2A, a ubiquitous form, is unexpectedly expressed.