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# COMMENTARY

# SEROTONECTIN AND THE FAMILY OF PROTEINS THAT BIND SEROTONIN

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Serotonin (5-hydroxytryptamine; 5-HT) is an extraordinarily active biogenic amine. In many ways it has proved to be a pharmacologist's delight, as the number of papers about it published since its discovery in 1948 [1] attest. Few tissues are unaffected by 5-HT. Among the systems that respond to 5-HT are the endocrine [2], nervous [3], and cardiovascular [4]. The plethora of actions that 5-HT has on systems such as these, that can so profoundly influence so many diverse bodily functions, makes 5-HT not only an interesting substance but a potentially dangerous one as well. Evolution appears to have preserved 5-HT to carry out a variety of roles in the physiology of several organ systems, but in doing so it has clearly protected organisms against the spectre of the significant release of free, active, circulating 5-HT. The danger of that eventuality is well illustrated by the phenomenon of lethal anaphylaxis in mice, a reaction characterized by vascular collapse and mediated by 5-HT [5].

Defenses against circulating 5-HT can be likened to American and Soviet nuclear policy. The governing principle of deterrence seems to be redundancy rather than sufficiency. 5-HT reaching the circulation is primarily inactivated by uptake and removal by the lung, a process in which the pulmonary endothelial cells play the critical role [6, 7]. Removal by the lung is backed up by hepatic metabolism [8, 9] and uptake into platelets [10]. In addition, a circulating protein has been found recently in the blood that has the remarkable property of being able both to bind 5-HT [11, 12] and to bind to cellular elements of the blood [13, 14]. This protein, serotonectin, is one of a family of different serotonin-binding proteins that are germ-layer specific. The recently discovered effectiveness of the type 2 (S2) 5-HT receptor antagonist ketanserin, as an anti-hypertensive agent [15, 16], suggests that a defect in the elaborate array of defenses against the circulation of free 5-HT may be involved in the pathogenesis of some types of hypertensive disease. This possibility, in turn, makes the family of 5-HT binding proteins of potential clinical as well as physiological interest.

The first 5-HT binding protein to be extensively characterized was found in homogenates of brain and called serotonin binding protein (SBP; [17–19]). The activity of this protein in brain and spinal cord regions parallels the concentration of 5-HT [20]. When lesions are made that obliterate the serotonergic neuronal cell bodies of the nuclei of the median raphe, SBP activity is lost from areas of the forebrain that receive serotonergic projections [20]. Moreover, lesions made through the descending serotonergic pathways to the spinal cord result in depletion of SBP below and accumulation of SBP above the lesions. These observations suggest that SBP is located within serotonergic neurons and that it reaches serotonergic terminal fields by axonal transport from its site of synthesis in cell bodies. Analysis of the rate of SBP accumulation above spinal cord transections indicates that SBP moves proximo-distally at a rate that is consistent with fast axonal transport. Fast axonal transport, in turn, is highly suggestive of a localization of SBP in synaptic vesicles because most of the material moving down axons at this rate is, in fact, vesicular (or at least sedimentable) and destined for axon terminals [21]. The hypothesis suggested by these data, that SBP is not only intraneuronal but a component of serotonergic synaptic vesicles, has been confirmed.

In subcellular fractionation studies of forebrain tissue, SBP is largely localized within the synaptosomal fraction [20]. If this fraction is rapidly lysed and reconstituted in $K^+$- and $Mg^{2+}$-containing buffer solutions [22], SBP is 4-fold more concentrated in the vesicles liberated from the synaptosomes than in the synaptosomal supernatant fraction [20]. SBP, moreover, is found in the serotonergic neurons of the gut as well as in those of the brain [23]. These peripheral enteric serotonergic neurons survive well *in vitro* and are ideal to use for studies of the stimulation-induced release of materials from nerve terminals [24]. Stimulation of enteric serotonergic neurons leads to a $Ca^{2+}$-dependent release of newly taken up exogenous [$^3$H]-5-HT [25], of endogenous 5-HT [26], and a parallel release of SBP as well [25]. When similarly stimulated, the enteric neurons do not release the cytosolic marker protein, lactic acid dehydrogenase. These last findings are consistent with the ideas that SBP is a component of the synaptic vesicles of serotonergic neurons, that these neurons release 5-HT by exocytosis, and that SBP and 5-HT are lost from vesicles as part of the exocytotic event.

If SBP is, in fact, a 5-HT vesicular storage protein, one might readily postulate that its function is to reduce intravesicular osmotic pressure. It has been estimated that the concentration of 5-HT within enteric serotonergic neurons, for example, is at least 7 mM [27]. Since it is likely that most intraneuronal

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5-HT will be found within synaptic vesicles, the intravesicular concentration of 5-HT must be even higher than that; nevertheless, the vesicles, despite their apparently high concentration of this small molecule, do not swell. If one molecule of SBP were to bind several of 5-HT, intravesicular osmotic pressure would be effectively reduced. Properties of SBP are ideally suited for such a role of the protein. SBP binds 5-HT with extremely high affinity in the presence of $Fe^{2+}$ and in $K^+$-buffers. The $K_D$ values are in the range of $10^{-10}$ M and $10^{-8}$ M [23]. On the other hand, in the presence of physiological concentrations of $Na^+$ and $Ca^{2+}$, SBP loses its affinity for 5-HT [28]. Within cells the dominant cation is $K^+$ and the concentrations of $Na^+$ and $Ca^{2+}$ are very low. In the extracellular fluid, however, these conditions are reversed; therefore, within synaptic vesicles SBP would be expected to tightly bind 5-HT but the 5-HT–SBP complex would be expected to dissociate when the vesicular interior becomes exposed to the extracellular fluid as occurs at the time of exocytosis.

Evidence derived from experiments in which $^3$[H]-5-HT was used as a probe to load synaptic vesicles of intact serotonergic neurons of brain or gut supports the idea that 5-HT is normally stored as a complex with SBP *in situ* [29]. There are two forms of purified SBP, one with an apparent molecular weight of 45 kilodaltons (kd) and one of 56 kd [30]. When neurons that have taken up [$^3$H]-5-HT are homogenized, protein bound [$^3$H]-5-HT can be isolated from the homogenate by gel filtration. This labeled complex migrates on sodium dodecyl sulfate (SDS) gels with apparent molecular weights of 45 kd and 56 kd. The 45 kd form predominates when the [$^3$H]-5-HT–SBP complex is formed from [$^3$H]-5-HT taken up by intact neurons while the 56 kd form is most abundant when [$^3$H]-5-HT is added to extracted SBP *in vitro* [29]. Since [$^3$H]-5-HT is largely taken up by 5-HT terminals when intact serotonergic neurons are exposed to this probe, it has been proposed that the 45 kd form of SBP is that found in terminal varicosities and the 56 kd material is derived from perikarya and non-terminal axons. In synaptosomes, and especially in vesicles, therefore, the ratio of the 45 kd to the 56 kd protein increases. This situation would imply that the 56 kd SBP may be the precursor of the 45 kd material, a proposition for which there is again supporting evidence.

SBP is a neurectodermal-specific protein. It has, therefore, been found only in the serotonergic neurons of the brain and gut [18, 19, 23, 25] and in the parafollicular cells of the thyroid gland [31, 32]. Central serotonergic neurons are neural tube derivatives while the enteric serotonergic neurons [33] and the parafollicular cells develop from the neural crest [34]. Other cells that are derived from different germ layers but which also store 5-HT do not contain SBP. Other 5-HT-storing cells include the enteroendocrine cells of the gastrointestinal mucosa [35], the mast cells of mice and rats [36], and platelets [10, 37]. Enteroendocrine cells are endodermal derivatives while mast cells and platelets come from mesoderm. These other cells do contain proteins that bind 5-HT; however, these proteins are specific for each cell-type and different from SBP. Mast cell [38] and enteroendocrine cell [39] proteins that bind 5-HT have been partially characterized and, like SBP, are probably intracellular proteins involved in the storage of 5-HT. In contrast, the analogous protein extracted from platelets, serotonectin, has been well characterized in rats and humans and is a surface component.

Serotonectin was originally identified in the 100,000 *g* supernatant fraction of homogenates of rat platelets [12, 13]. It was found to be a glycoprotein that binds 5-HT with high affinity ($K_D$ values of 42 nM and 0.8 $\mu$M). The glycoprotein has an apparent molecular weight, 200 kd, that is similar to that of fibronectin, but serotonectin is distinguishable from fibronectin. Serotonectin has been purified, and monospecific antibodies have been raised against it [13]. When used for the light and electron microscopic immunocytochemical detection of rat serotonectin, these antibodies revealed that all serotonectin-like material is located on rat platelet surfaces. No serotonectin immunoreactivity can be found within platelets or on other cells of the peripheral blood of the rat [13]. This indicates that serotonectin is a membrane protein; however, it appears to be a peripheral glycoprotein adsorbed to the external face of the platelet plasma membrane, not an integral protein. Washing platelets with Krebs solution removes most of the extractable serotonectin from them. Moreover, the circulation of non-platelet bound, free serotonectin in the blood can also be demonstrated. Antiserum to serotonectin does not cross-react with SBP, and no serotonectin can be found in (or on) brain synaptosomes. It seems likely, therefore, that rat serotonectin is a circulating glycoprotein that binds 5-HT on the one hand and to platelet membranes on the other and is probably in equilibrium in the blood between platelet-bound and unbound forms.

Immunoprecipitation of newly-synthesized radioactive serotonectin has revealed that the glycoprotein is synthesized in blood-forming organs. These organs include, in the rat, the hematopoietic liver and spleen as well as the bone marrow, but not the mucosa of the gut. Rat serotonectin may play a role in the ability of platelets to take up 5-HT because anti-serotonectin antibodies inhibit platelet uptake of [$^3$H]-5-HT; however, serotonectin is probably not the membrane 5-HT transport molecule itself. The glycoprotein does not bind a probe for the uptake site, [$^3$H]-imipramine [13, 40], and removal (by washing) of serotonectin from rat platelet membranes does not reduce the binding of [$^3$H]-imipramine by platelets. Certainly, therefore, an action on serotonectin cannot be invoked to explain the ability of imipramine to impede rat platelet 5-HT uptake.

Human blood, like that of the rat, contains a protein that circulates, binds to the surface of platelets, and also binds 5-HT with high affinity and specificity. This protein has some properties that are different from the analogous protein of rats but, because of the essential similarities between the human and rat substances, the human protein has been called human serotonectin [14]. Human serotonectin, like that of rats, is a glycoprotein. It has been purified and found to have a molecular weight

of about 200 kd. Binding constants for 5-HT are 36 nM and 1.1 μM. Antibodies to rat serotonectin do not react with the human material. Correspondingly, monospecific antibodies raised against human serotonectin do not recognize rat serotonectin. The primary structures of the two proteins, therefore, are probably different. The antisera that have been raised in rabbits against human serotonectin have been used both to locate the glycoprotein and to quantitatively estimate it.

Human serotonectin, in analogy with the rat material, probably circulates in the plasma and binds to the outer faces of the plasma membranes of cellular elements of the blood. It binds, however, to more cells of the blood than does the serotonectin of rats. Human serotonectin, as does human fibronectin [41, 42], binds to the surfaces of both granulocytes and lymphocytes as well as platelets. It does not, however, bind to red blood cells. Human serotonectin-like material can also be found immunocytochemically in association with developing cells in smears of bone marrow.* It can be demonstrated on megakaryocytes and on the precursors of eosinophils. It is not yet clear that either of these cell types actually synthesize human serotonectin; however, the observation that the protein is found in or on some bone marrow cells is consistent with the hypothesis that human, like rat serotonectin, is produced in hematopoietic tissue.

Human serotonectin can be removed completely from the membranes of white blood cells by washing them with isotonic solutions of sucrose or salts. This treatment is less effective in removing human serotonectin from the plasma membranes of platelets. About 25% of the platelet-bound human serotonectin resists removal by washing in physiological solutions. It seems likely, therefore, that human serotonectin binds more tightly to platelets than it does to white blood cells. Since megakaryocytes do contain human serotonectin-like immunoreactivity, it is conceivable that this cell synthesizes the substance and that the residual human serotonectin, not removed from platelet membranes by washing, is an integral membrane protein. This point remains to be established but it is possible that circulating human serotonectin is not secreted from its cell of origin by exocytosis but instead may be clipped from platelet surfaces. This idea is, of course, highly speculative, but one could envision a mechanism whereby human serotonectin is originally produced within megakaryocytes (and/or eosinophilic precursor cells) as an integral membrane protein. The glycoprotein, according to this hypothesis, might have a long sequence that protrudes externally from the plasma membrane. Circulating human serotonectin may arise by proteolytic cleavage of the protruding chain of the molecule, leaving a reversible binding site for the cleaved protein on the cell surface. This type of mechanism is similar to that which results in sucrase-isomaltase activity of the brush border of enterocytes of the small intestine [43]. Sucrase-isomaltase is made as a long integral membrane protein. Proteolytic cleavage at the cell surface nicks sucrase from the large molecule. Isomaltase remains anchored in the brush border membrane, and the sucrase subunit becomes attached by tight intramolecular binding to the isomaltase.

The function of human serotonectin is uncertain. Anti-human serotonectin antibodies antagonize the uptake of 5-HT by human platelets [14] but they do not inhibit the 5-HT-induced aggregation of platelets [44, *]. Human serotonectin, therefore, may play a role in the platelet 5-HT uptake mechanism. It seems noteworthy, in this regard, that anti-human and anti-rat serotonectin antibodies both inhibit uptake of 5-HT by the platelets of the respective species. The function, if any, of human serotonectin bound to human white blood cells, however, is obscure. It should also be kept in mind that endothelial cells are among the potential targets of 5-HT. The amine causes inter-endothelial cell junctions, particularly of post-capillary venules, to separate. This vastly increases vascular permeability [45]. It has been proposed that this action of 5-HT may be important in permitting effector cells to leave the circulation during delayed-type hypersensitivity reactions [46, 47]. This reaction and that of vascular smooth muscle may be modulated and localized by the binding of 5-HT to circulating serotonectin.

In summary, 5-HT has been found to act on many targets in a variety of tissues and organs. The effects of the amine are profound, widespread, and potentially dangerous. Storage and release of 5-HT are, therefore, important functions that are rigorously regulated. In addition, the circulation of free 5-HT seems to be distinctly limited so that its action, when it is released, remains localized. A family of proteins that tightly and specifically bind 5-HT appears to act as carriers for the amine and may be important in these processes. One protein that seems to play a role in intracellular storage of 5-HT is the neurectodermal protein, SBP. Another protein, of potentially great clinical significance, is the glycoprotein, serotonectin. This latter material, found thus far in rats and humans, may limit the vascular reactivity of 5-HT and function in bringing 5-HT to platelets and may also participate in the platelet 5-HT uptake mechanism.

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