The signal transducing system coupled to serotonin-S_2 receptors

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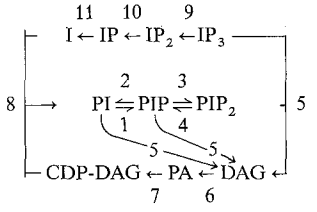
Summary. The signal transducing system coupled to the serotonin-S_2 receptor on platelets involves metabolism of inositol-containing phospholipid, elevation of intracellular free Ca^{2+} and activation of protein kinase C. Evidence for coupling of the serotonin-S_2 receptor to the same signal transducing system in brain and smooth muscle tissue is reviewed.
Key words. Serotonin-S_2 receptor; signal transduction; phosphoinositides.

The classification of serotonin receptors is largely based on data from radioligand binding studies. The 5-hydroxytryptamine$_1$ (5-HT_1) or serotonin-S_1 sites have high (nM) binding affinity for agonists and a low (μM) affinity for most serotonin antagonists. In contrast, the 5-HT_2 or serotonin-S_2 sites reveal nanomolar binding affinity for serotonin antagonists and micromolar affinity for serotonin. Based on displacement studies with specific drugs, serotonin-S_1 (5-HT_1) sites have been further subclassified in serotonin-S_{1A} (5-HT_{1A}), S_{1B} (5-HT_{1B}) and S_{1C} (5-HT_{1C}) sites (table). The functional correlates designated to these sites are still speculative. In contrast, roles for the serotonin-S_2 sites were demonstrated in serotonin agonist induced behavioral excitation and discriminative stimulus effects, in serotonin-induced smooth muscle contraction in blood vessels, trachea, uterus and ileum, and in functional investigations on blood platelets[18]. Moreover, ritanserin, a specific and long-acting serotonin-S_2 antagonist was found to double slow wave sleep in volunteers and the drug has beneficial effects in dysthymic disorders and on negative symptoms in schizophrenia[15, 22]. At the biochemical level a relationship between S_1-sites and serotonin-stimulated adenylate cyclase has been suggested[2], but ample evidence refuted this hypothesis. Until a few years ago evidence for the involvement of phospholipid turnover in the signal transducing system coupled to serotonin receptors was rather poor. Earlier studies on various tissues[3, 14, 16, 19] have demonstrated that serotonin altered the incorporation of [^{32}P] phosphate in phosphatidylinositol (PI) and phosphatidic acid (PA) during long-term incubations (30–120 min). Studies on blowfly salivary glands[4] illustrated a rapid breakdown of the polyphosphoinositides phosphatidylinositol 4,5-bisphosphate (PIP_2) and phosphatidylinositol 4-phosphate (PIP). However, the type of receptor site which is involved, has not been characterized. In recent years, knowledge on the receptor-coupled signal transducing system involving inositol phospholipid metabolism has rapidly progressed. As an initial biochemical step the activation of a PIP_2 specific phospholipase C was hypothesized yielding diacylglycerol and inositoltrisphosphate (IP_3) (fig.). Both reaction products are suggested to act as second messengers by provoking the activation of the protein kinase C and the release of Ca^{2+} from intracellular stores, respectively[5, 20]. Diacylglycerol is rapidly phosphorylated to PA; IP_3 is phosphorylated to IP_4 and both are subsequently dephosphorylated to inositol, which is reincorporated in the phospholipids.



Inositol phospholipid metabolism. PIP_2: Phosphatidylinositol 4,5-bisphosphate; PIP: Phosphatidylinositol 4-phosphate; PI: Phosphatidylinositol; DAG: Diacylglycerol; PA: Phosphatidic acid; IP_3: Inositoltrisphosphate; IP_2: Inositol diphosphate; IP: Inositol phosphate; I: Inositol; 1: PI kinase; 2: PIP phosphomonoesterase; 3: PIP kinase; 4: PIP_2P phosphomonoesterase; 5: phospholipase C; 6: DAG kinase; 7: CTP-phosphate cytidyltransferase; 8: CDP:1,2-DAG inositol phosphatidyltransferase; 9, 10, 11: Inositol phosphate phosphomonoesterases.

Identification of the signal transducing system coupled to serotonin-S_2 receptors in human platelets

In studies using human platelets we provided ample evidences that serotonin-induced phospholipid metabolism was directly coupled to serotonin-S_2 receptor stimulation[11, 12]. Addition of serotonin to human platelets, prelabeled to isotopic equilibrium with [^{32}P] orthophosphate was found to provoke a) an increase in myosin light chain phosphorylation, which is a measure for the mobilization of Ca^{2+}, b)

Subclassification and characteristics of serotonin receptor binding sites

Type of binding site	Typical labeled ligand	Enriched tissue	Proposed roles
5-HT_{1A}	[^{3}H]—N, N-dipropyl-8-hydroxy -2-aminotetralin (8-OHDPAT)	Hippocampus	*Behavioral syndrome (excitation) Body temperature Food intake *Dendritic autoreceptor Vascular smooth muscle contraction
5-HT_{1B}	^{125}I-cyanopindolol	Cortex	*Behavioral syndrome (inhibition locomotion) *Peripheral presynaptic function
5-HT_{1C}	[^{3}H]-5-HT/mesulergine	Choroid plexus	*Cerebrospinal fluid productions
5-HT_2	[^{3}H]-ketanserin	Frontal cortex	Behavioral syndrome (excitation) Discriminative stimulus effects Smooth muscle contraction Platelet function Slow wave sleep Dysthymic disorders

*The involvement of the sites in these functions are still hypothetical.

an increase in phosphorylation of the 40 kDa protein, a measure for the activation of the protein kinase C, and c) PA formation, a measure for phospholipase C activity. More recently we could demonstrate serotonin-induced formation of diacylglycerol and inositolphosphates concomitant with a decrease in PIP_2 content. PIP was found to be synthetized but not by light de novo light synthesis, PI levels did not change but the PI cycling increased [11–13]. All these alterations reached their maximal level within seconds after addition of the agonist, a prerequisite for their involvement in signal transduction.

Evidence that the receptor involved was of the S_2 type was provided from the agonist dose response relationship [12]. The ED_{50} for serotonin-induced PA formation corresponded to the K_i-value of serotonin for S_2-receptor sites measured with [^{3}H]-ketanserin on frontal cortex and cat platelet membranes and with [^{3}H] LSD on human platelet membranes. Furthermore the potency of 13 drugs to antagonize the serotonin-induced PA formation was virtually identical to their binding affinity for serotonin-S_2 receptor sites [12]. Similar close correlations were demonstrated with drug potencies to inhibit serotonin-induced platelet aggregation [12].

The serotonin-S_2 receptor coupled signal transducing system in brain tissue

Investigations on serotonin-induced inositol phospholipid metabolism in rat brain slices showed that the situation in this tissue was much more complex [7, 17]. In these studies the formation of water-soluble [^{3}H]-inositolphosphates was measured after relatively long-term incubation (45 min) of [^{3}H]-inositol loaded slices with serotonin. Using slices from rat cortex, Kendall and Nahorsky [17] found a biphasic increase in inositol phosphate formation with increasing serotonin concentration. The so-called 'high affinity response' (at micromolar serotonin) was found to be decreased following chronic treatment of rats with antidepressants and following treatment of the frontal cortex tissue with phenoxybenzamine. These treatments also caused a reduction in B_{max}-value of [^{3}H]-ketanserin binding, but to a somewhat lesser extent. Based on these findings the authors suggested a partial coupling of frontal cortical serotonin-S_2 receptors to phospholipid turnover. The so-called 'low affinity' response to millimolar concentrations of serotonin was not inhibited by ketanserin and a correspondence with a specific receptor site was not evident. Conn and Sanders-Bush [7] observed a serotonin-induced increase in inositolphosphate formation in slices of various brain areas. However, only in slices from the frontal cortex the serotonin-induced response was inhibited by serotonin antagonists. The inhibitory potencies of the drugs were 100-fold weaker than their binding affinities for serotonin-S_2 receptor sites but there was a correspondence in rank order of potency. In more recent studies [8], this group estimated the Kd-values by Schild analyses of the effects of different antagonists on serotonin-induced inositol phospholipid response. Although the Kd-values were higher than those determined in radioligand binding assays, regression analysis demonstrated a good correlation. Hence, a link between serotonin-S_2 receptor sites with frontal cortex and serotonin-induced phospholipid metabolism was suggested. The most prominent increase in serotonin-induced [^{3}H]-inositolphosphates formation was however found in the slices from the choroid plexus [9]. In this tissue serotonin was 10-fold more potent than in the cortex; the potency of different drugs to antagonize the phospholipid response were apparently consistent with binding to the serotonin-S_{1C} site.

As suggested by Kendall and Nahorsky [17] and Conn and coworkers [7] a possible explanation for the discrepancy between the radioligand binding and the biochemical data might be the difference in experimental preparation when using membranes and slices. Indeed in contrast to cell suspensions the slow diffusion rate of drugs in tissue slices does not allow to measure synchronous stimulation of the cells. The possibility that the inner part of the slices becomes anoxic which might affect energy-dependent processes as the inositol lipid metabolism, should also be considered.

As employed by both groups the measure of agonist-induced release of water soluble inositolphosphates from [^{3}H]-inositol prelabeled tissue might be another source of error. Although on theoretical grounds this method looks sound and in practice it is more convenient than phospholipid analyses, it should be kept in mind that only water-soluble inositol phosphates are measured. After prolonged incubation with the agonist, the release of [^{3}H]-inositolphosphates from the [^{3}H]-inositol-labeled tissue does not necessarily reflect a direct coupling of the serotonin-S_2 receptor to inositol-phospholipid breakdown. Furthermore, metabolic alterations other than the hypothesized increase in phosphoinositide-specific phospholipase C activity might be the cause of the increase in [^{3}H]-inositolphosphates. These can be inositolphospholipid synthesis or a non-specific phosphodiesteratic cleavage of phospholipids. Although the evidence is accumulating that in many cell types receptors transduce their signal by phosphodiesteratic cleavage of inositol-containing phospholipids, we believe more effort should be put into the identification of different metabolic steps involved. Differences in receptor-coupled phospholipid response between tissues and receptors that might not be apparent by quantifying inositolphosphate release after receptor activation, should also be considered as a possible source of error when compared to radioligand binding data.

Brain slices do contain different cell types. Recently convincing evidence was presented for the coupling of a serotonin receptor on glial cells to the signal transducing system involving inositol-phospholipid metabolism [1, 21]. Using C6 glioma cells, Ananth and coworkers [1] found that serotonin stimulated the inositol phospholipid cycling with an EC_{50} of 1.2×10^{-7} M; a value close to 2×10^{-7} M we found in the platelets. Serotonin-induced changes occurred immediately after the addition of the agonist. In view of the potency by which various drugs inhibited the phospholipid response, it was concluded that the serotonin-S_2 receptor was involved. Ogura and coworkers [21] found that stimulation of a clonal cell line of glial origin with serotonin, induced an elevation of intracellular Ca^{2+}. This response was antagonized by serotonin-S_2 antagonists. Since Ca^{2+} is an intracellular messenger after activation of receptors that are coupled to inositol phospholipid breakdown, the serotonin receptors involved in this study are likely of the S_2-type and coupled to inositide breakdown; the involvement of S_{1C}-receptor sites can, however, not be excluded. In primary cultures of cerebellum granule cells, serotonin was reported [24] to induce an increase in [^{3}H]-inositolphosphate in a dose-dependent manner with an ED_{50}-value of 10^{-7} M. This response was also potently blocked by serotonin-S_2 antagonists.

Serotonin-S_2 receptors in smooth muscle

Evidence for the coupling of serotonin-S_2 receptors in other tissue besides platelets and brain is scarce. Serotonin-S_2 receptor stimulated inositol phosphate production was found in rat aortic myocytes [10], in rat thoracic aorta rings [23] and in the isolated rat tail artery [6]. Using the ^{32}P-labeled rat aorta cell lines A_7A_5 and A_{10} we found that serotonin provoked essentially the same alterations in phospholipid metabolism as in platelets that could be antagonized by nM doses of ketanserin (unpublished results).

Conclusion

It can be stated that the signal-transducing system coupled to the serotonin-S_2 receptor involves inositol phospholipid breakdown. As far as we know, only for human platelets have the metabolic alterations in phospholipids, protein phosphorylation and Ca^{2+} metabolism been identified. The primary step (possible coupling by nucleotide-binding proteins and/or the first enzymatic step which becomes activated) remains to be elucidated. A more profound study of the metabolic steps involved in the phospholipid metabolism on activation of serotonin-S_2 receptors in other tissue is necessary to evaluate whether the same metabolic steps occur in the different tissue.

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Defective phosphoinositide metabolism in primary hypertension

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Summary. An increase in free cytosolic calcium content has been reported in essential hypertension. Since within the membrane, the phosphoinositides participate in the control of cell calcium homeostasis, we investigated whether impaired phosphoinositide metabolism could account for the calcium handling abnormality observed in hypertensives. In erythrocyte membranes of hypertensives the activity of kinases involved in polyphosphoinositide formation appears to be impaired and could be related to the alteration in calcium binding capacity and ATP-dependent calcium transport. In platelets of hypertensives, the hyperactivity of phospholipase C (observed even in the absence of calcium in the external medium) is likely to be responsible for the hypersensitivity of cells to various agonists. These observations are consistent with the hypothesis that in cells from hypertensives, a membrane defect linked to phosphoinositide metabolism is involved in the overall calcium handling defect.
Key words. Red blood cell; platelet; phospholipids; phospholipase C.

The increase in peripheral resistance that characterizes the established phase of primary hypertension results from an increased active tension in the vascular smooth muscle and this is likely to be a reflection of an increased concentration