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DEVELOPMENTALLY REGULATED SEROTONIN 5-HT_{2B} RECEPTORS

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Serotonin (5-hydroxytryptamine, 5-HT) binds to numerous cognate receptors to initiate its biological effects. In this review, we have focused on the 5-HT_{2B} receptor to address how signaling and expression of this receptor is specifically implicated in embryonic development and adult health and disease. Transduction of the 5-HT_{2B} signaling is complex, including phospholipase C and A₂ stimulation, cGMP production and a mitogenic signal that integrates the tyrosine kinase signaling pathway. Furthermore, 5-HT, through the 5-HT_{2B} receptors, has the ability to control serotonergic differentiation of committed neuron-like cells. In addition, 5-HT_{2B} receptors are actively involved in the transient action of 5-HT during embryonic morphogenesis. Our recent data presented the first genetic evidence that 5-HT via 5-HT_{2B} receptors regulates cardiac embryonic development and adult functions and suggested that this receptor subtype may be involved in other physiopathological situations. In particular, 5-HT-dependent molecular mechanisms may be involved in embryonic development and postnatal maturation of the enteric nervous system. Also, the involvement of the 5-HT_{2B} receptor in the vascular growth often observed in hypertension is likely. These probably result from reactivation of developmentally regulated receptors in pathological situations. Finally, embryonic functions of 5-HT₂ receptors observed in *Drosophila* gastrulation suggest evolutionary conserved mechanisms.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT), first isolated as a vasoconstrictor from blood in 1948 by Page and co-workers, was later identified in the central nervous system (CNS). It is found in three main areas of the body: the intestinal wall (where it causes increased gastrointestinal motility), blood vessels (where large vessels are constricted), and the CNS. Although 5-HT may be obtained from a variety of dietary sources, it is also endogenously synthesized from tryptophan through the actions of the enzymes tryptophan hydroxylase and aromatic L-amino acid decarboxylase. Both dietary and endogenous 5-HT are rapidly metabolized by monoamine oxidase. The functions of 5-HT are numerous and appear to involve the control of appetite, sleep, memory and learning, temperature regulation, mood, sexual and hallucinogenic behavior, cardiovascular function, muscle contraction, endocrine regulation, and depression. Peripherally, 5-HT appears to play a major role in platelet homeostasis, blood pressure regulation, cardiac contractility, motility of the gastro-intestinal tract, and carcinoid tumor secretion ¹. Of the neurotransmitter substances, 5-HT is perhaps the most implicated in the etiology of various disorders, including anxiety, depression, obsessive-compulsive disorder, schizophrenia, stroke, obesity, pain, hypertension, vascular disorders, migraine, and nausea.

5-HT binds to numerous cognate receptors to initiate its biological effects. There are at least 15 different 5-HT receptors that belong to four families: 5-HT_{1/5}, 5-HT₂, 5-HT₃, and 5-HT_{4/6/7} ². All but 5-HT₃ belong to the G-protein coupled receptor superfamily and have seven transmembrane domain structures. Distinct subtypes of receptors exist: The 5-HT₂-receptor family comprises three closely related subtypes: 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}. All 5-HT₂ receptors show similar protein sequences with homology varying from 70 to 95%. However, these subtypes are differentially expressed in a time and tissue specific manner. In this review, we have focused on the 5-HT_{2B} receptor to address how signaling and expression of this receptor is specifically implicated in embryonic development and adult, health and disease.

Coupling of mammalian 5-HT_{2B} receptors

The main transduction pathway for the family of 5-HT₂ receptors is the activation of phospholipase C second messenger via the alpha subunit of the Gq GTP binding protein. This messenger activates inositoltrisphosphate and diacylglycerol production leading to Ca²⁺ release and protein kinase C activation ³ (Figure 1A). In addition, 5-HT_{2B} receptor stimulation has the ability to activate the arachidonic acid pathway via phospholipase A₂ ⁴ and the nitric oxide synthesis pathway via a PDZ type I C-terminal motif ⁵. In the absence of growth factors (serum free), agonist stimulation of 5-HT_{2B} receptor subtype also causes a rapid and transient activation of the proto-oncogene product p21^{ras} ⁶. Activation of 5-HT_{2B} receptors is mitogenic and leads to cell cycle progression through p42^{mapk}/p44^{mapk} (ERK₂/ERK₁) mitogen-activated protein kinases (MAPK). In this mitogenic signaling, c-Src and PDGF-receptor tyrosine kinase activities

are also stimulated ⁷ leading to retinoblastoma protein hyperphosphorylation, and rapid activation of both cyclin D1/cdk 4 and cyclin E/cdk 2 kinases by a mechanism which is dependent upon increase of cyclin D₁ and cyclin E protein levels. The induction of cyclin D₁ expression, but not that of cyclin E is under MAPkinase control, indicating an independent regulation by 5-HT. Activation of the 5-HT_{2B} receptor transactivates tyrosine kinase activity of Src that is absolutely necessary to trigger both PDGF-receptor/MAPkinase-dependent (cyclin D₁) and -independent (cyclin E) cell cycle progression ⁷. These observations suggest that 5-HT_{2B} signaling is more complex than previously thought and that it integrates the tyrosine kinase signaling pathway.

During serotonergic *in vitro* differentiation, the 5-HT_{2B} receptor is specifically expressed

The 5-HT_{2B} receptor can control differentiation of embryonic carcinoma cells by cross talk with other subgroups of 5-HT receptors. 1C11 cells, an immortalized embryonic carcinoma cell line, can be differentiated into serotonergic or catecholaminergic cells by cyclic AMP (cAMP) treatment ⁸⁻¹⁰. The switch from the undifferentiated committed 1C11 cell type to the 1C11* serotonergic cells occurs within 4 days of cAMP treatment. In addition to the onset of 5-HT metabolism, storage, and transport, 1C11* cells also sequentially express 5-HT_{1B/1D}, 5-HT_{2B}, and 5-HT_{2A} receptors within this period. 5-HT_{2B} ³ and 5-HT_{1B/1D} receptors are expressed after 2 days of cAMP treatment (1C11*d2) while 5-HT_{2A} receptors are only detected after 4 days of treatment ¹¹ (1C11*d4) (Figure 1B). 5-HT_{2B} and 5-HT_{2A} receptors are able to stimulate phospholipase C in this cell system. The 5-HT_{2B} receptors stimulate the phospholipase A₂-mediated release of arachidonic acid (AA) in 1C11*d2 cells, but inhibit the 5-HT_{1B/1D} receptor-dependent cAMP inhibition via a cyclooxygenase-dependent AA metabolite. By contrast, in 1C11*d4 cells, this 5-HT_{2B}-mediated inhibition of the 5-HT_{1B/1D} function becomes ineffective upon concomitant 5-HT_{2A} activation. The signaling pathway of 5-HT_{2A} receptors interferes, thus, with that of 5-HT_{2B} receptors on the transduction of 5-HT_{1B/1D} receptors ⁴. Furthermore, the 1C11*d4 cells express a functional 5-HT transporter and external 5-HT during differentiation reduces the maximal velocity of the uptake ¹². Therefore, 5-HT has the ability to control serotonergic differentiation of these cells, through the 5-HT_{2B} receptors.

Expression of 5-HT_{2B} receptors

Specificity of the 5-HT_{2B} functions could also be related to tissue specific expression. The adult rat 5-HT_{2B} receptor expression, first restricted to the stomach fundus ^{13, 14}, was subsequently found in the brain, more specifically in the cerebellum, cortex, hippocampus and amygdala ¹⁵, similar locations express 5-HT_{2B} receptor in the mouse brain ¹⁶. The 5-HT_{2B} receptors are also found in the adult gut and cardiovascular system ¹⁷, including the stomach, intestine and pulmonary smooth muscle as well as in

pulmonary and vascular endothelial cells and cardiomyocytes in mouse¹⁶, human¹⁸⁻²⁰ and rat^{13, 14, 21, 22}.

In mouse, 5-HT has been shown to participate in craniofacial and cardiac morphogenetic events^{23, 24}. Strikingly, we observed that the 5-HT_{2B} receptor is expressed in embryos with a peak of expression at 8.5 days post-coitum in mice²⁵, whereas there were only low levels of 5-HT_{2A} and no 5-HT_{2C} receptor mRNA expression at this stage^{25, 26}. A peak of binding on a membrane preparation from 9 day post-coitum embryos was also observed. In addition, by whole mount *in situ* hybridization and immunohistochemistry, we detected 5-HT_{2B} expression in neural crest cells, heart myocardium, and somites. The requirement for functional 5-HT_{2B} receptors between 8 and 9 day post-coitum is supported by the culture of embryos exposed to 5-HT₂-specific ligands; 5-HT_{2B}-high affinity antagonists such as ritanserin, induce morphological defects in the cephalic region, heart and neural tube²⁵. These antagonistic treatments interfere with cranial neural crest cell migration and in the heart, are responsible for premature sarcomeric organization of the subepicardial layer and for the absence of the trabecular cell layer in the ventricular myocardium. These observations suggested strongly that 5-HT_{2B} receptors are actively involved in a transient action of 5-HT during embryonic morphogenesis.

Murine cardiac development involves 5-HT_{2B}

Our *in vivo* study using mice carrying an inactivating mutation in the 5-HT_{2B} receptor gene generated by the gene targeting technique (homologous recombination) confirmed the embryonic functions of 5-HT_{2B} receptors²⁷. We have shown that the lack of 5-HT_{2B} receptors leads to disturbed heart development. Severe heart abnormalities, including trabecular defects (Figure 2A-B) account for the mid-gestation lethality. Remarkably, the defects noted in 5-HT_{2B} mutant hearts, are reminiscent of those seen in mice lacking neuregulin or its receptors ErbB-2 and ErbB-4. In 5-HT_{2B} receptor mutant embryos, a specific reduction of ErbB-2 expression and of its effectors was observed. These results support the notion that the signaling pathway of tyrosine kinase receptors uses the Gq-coupled receptor 5-HT_{2B} for cardiac differentiation²⁷.

We have also observed cardiac histopathological changes including myocytes disarray and ventricular dilation in surviving 5-HT_{2B} mutant mice. These result from a decrease in size in mutant newborn myocytes which is accompanied by decreased cell density due to impaired proliferation. Moreover, the neonatal mutant heart developed major defects in cytoarchitectural organization. Interestingly, N-Cadherin expression is severely reduced in the mutant heart and intercellular junctions (intercalated disc) display strong ultrastructural abnormalities (to be published elsewhere). 5-HT_{2B} receptor seems, therefore, to be required for cytoskeleton assembly of membrane structures by regulating the N-cadherin expression within the intercalated discs. The impaired intercellular intercalated disc structures observed in the 5-HT_{2B} mutant mice heart could be the basis of impaired contractility and myofibrillar degeneration.

These data represent the first genetic evidence that 5-HT via 5-HT_{2B} receptor regulates cardiomyocyte embryonic development and adult functions suggesting that this receptor subtype may be involved in other physiopathological situations.

Implication of 5-HT_{2B} receptors in hypertension

Elevated arterial pressure or hypertension affects 15 to 20% of the adult population in industrialized societies and is one of the major risk factors for stroke, myocardial infarction and renal disease. The balance between contractile and relaxant factors defines the blood vessel pressure. 5-HT stimulates both contraction and relaxation of blood vessels, depending on the receptor subtypes localized in different blood vessels layers. Activation of 5-HT_{2B} receptors expressed on the endothelium relaxes the vessels through stimulation of nitric oxide release^{5, 28}. In smooth muscle cells, a 5-HT_{2A} receptor population primarily mediates 5-HT-dependent contraction in arteries from normotensive rats. Recently, Watts and colleagues presented evidence that a receptor population with 5-HT_{2B} pharmacology primarily mediates arterial contraction in deoxycorticosterone acetate-salt hypertension²⁹⁻³¹. Moreover, an increase in 5-HT_{2B}-receptor activation participates in maintaining high blood pressure in deoxycorticosterone acetate-salt hypertension since a 5-HT_{2B}-receptor antagonist such as the recently developed LY-272015 (5-HT_{2B}-specific) reduces elevated blood pressure³². Therefore, the ratio of 5-HT_{2B} receptor expression in endothelial cell versus vascular smooth muscle cells of blood vessels could be important in hypertensive situations. Since the 5-HT_{2B} receptor has a obvious implications in the mitogenic signaling of 5-HT, it could be involved in the vascular growth often observed in hypertension. Circulating levels of 5-HT could be increased in hypertension since a decreased uptake of 5-HT in platelets (main storage source for circulating 5-HT) from hypertensive patients has been established³³. The switch in receptor expression from the 5-HT_{2A} to the 5-HT_{2B} receptor and an increase in circulating levels of 5-HT seem important for hypertension pathology.

Developmental role of 5-HT_{2B} receptors in enteric neurons

The enteric nervous system, part of the peripheral nervous system, arises from restricted levels of the neural crest. Signals that influence the fate of crest-derived neural and glial precursors include glial cell line derived neurotrophic factor, neurotrophin-3, neuropoietic cytokines, endothelin-3, and laminin-1³⁴. Early-and late-developing lineages of enteric neuronal precursors, distinguished by dependence on expression of the *mash-1* gene, have been identified. All enteric serotonergic neurons are *mash-1*-dependent and develop early. Thus, 5-HT may influence the development of late-developing neurons since some enteric neuronal progenitors express 5-HT receptor(s). One such receptor, 5-HT_{2B}, is observed to be highly and transiently expressed in primordial enteric ganglia³⁵. 5-HT enhances enteric neuronal development *in vitro* by stimulating 5-HT_{2B} receptors. The appearance of the 5-HT_{2B}

receptor coincides with that of serotonergic neurons and *mash-1*-independent neurons. The transient expression of the 5-HT_{2B} receptor mediates serotonergic effects on the development of the late-developing, *mash-1*-independent set of enteric neurons. Active 5-HT uptake and synapses are formed between early-developing enteric neurons and still-dividing neuronal precursors in the fetal myenteric plexus³⁶. 5-HT-containing enterochromaffin cells, as well as serotonergic neurons are present when 5-HT_{2B} receptors appear in the gut. 5-HT via 5-HT_{2B} acts, therefore, on cells in the neuronal lineage to promote the development of enteric neurons³⁵. The timing and the pattern of the expression of this receptor in the bowel, as well as the development of enteric sources of 5-HT suggest that late-developing (*mash-1*-independent) neurons are the putative targets of 5-HT. This possibility is under investigation in 5-HT_{2B} mutant mice. Similar 5-HT-dependent molecular mechanisms may be involved in CNS embryonic development and/or its postnatal maturation.

Developmental functions of 5-HT receptors in other species

Independently of its location in adults, 5-HT has also been detected during zygotic cleavage divisions, gastrulation and neurulation in embryos of sea urchins, frogs and chickens. The presence of 5-HT and 5-HT receptors early in embryogenesis and the ability of 5-HT-specific pharmacological agents³⁷ to interfere with embryonic development suggests that early embryos use 5-HT to regulate cell proliferation and/or morphogenetic movements during neurogenesis³⁸. However, the 5-HT receptors and their molecular effectors involved in development remain unresolved^{39, 40}.

In insects, biogenic amines, including 5-HT, have been implicated as neuromodulators and neurotransmitters⁴¹ in addition to their function of cross-linking proteins and chitin during sclerotization of the cuticle⁴². In *Drosophila melanogaster*, a detailed immunohistochemistry of 5-HT localization in the brain has been described⁴³. In *Drosophila*, 5-HT regulates salivary gland secretion, heart and oviduct contractions, circadian rhythms, and influences learning and memory⁴¹. The G-protein coupled receptor 5-HT_{2Dr0} is orthologous to the mammalian 5-HT₂ receptor subfamily⁴⁴. 5-HT_{2Dr0} was observed to be highly expressed and developmentally regulated: The 5-HT_{2Dr0} mRNA expression starts early during embryogenesis, at the onset of gastrulation, with a surprising pattern that is similar to that of the pair-rule class of segmentation genes, *fushi-tarazu*. The transient expression of this receptor is restricted to the presumptive ectoderm and the corresponding peak of binding sites coincides precisely with the initiation of the rapid phase of ectoderm extension and with a transient wave of 5-HT synthesis⁴⁵. Ectoderm extension is delayed in *Drosophila* gastrulae devoid of the 5-HT_{2Dr0} receptor and this abnormal ectodermal elongation results in embryonic lethality. Associated with this desynchronization, there is an altered distribution of E-cadherin based adherens junctions in the ectoderm (Figure 2C-F) suggesting a function for 5-HT_{2Dr0} in ectodermal cell adhesion. Thus, 5-HT controls intercalation movements during germband extension⁴⁶.

During *Drosophila* gastrulation, the process of ectoderm extension is homologous to that of convergent extension responsible for elongation of the archenteron during sea urchin gastrulation⁴⁷. Interestingly, in sea urchin gastrulae, 5-HT has been reported to regulate blastopore invagination leading to archenteron elongation^{48,49}. Similarly, 5-HT antagonists have been shown to affect cell movements during neuroepithelium convergent extension at the neurulation stage in chicken⁵⁰.

Conclusions

Induction of receptors in pathological states is a concept which has recently been receiving greater attention⁵¹. The implication of 5-HT signaling via the 5-HT_{2B} receptor is now demonstrated in murine cardiac development and *Drosophila* gastrulation. It remains to be firmly established in mammalian brain maturation and enteric development. Associated with the abnormal gastrulation movements in *Drosophila* embryos lacking 5-HT_{2Dr0} receptors, there is an altered distribution of E-cadherin based adherens junctions in ectodermal cells. Similarly, N-Cadherin expression is also severely reduced in the heart of homozygous 5-HT_{2B} mutant mice and intercellular junctions display strong ultrastructural abnormalities. These similarities suggest that evolutionary conserved mechanisms are used by these orthologous receptors. In addition, the signaling pathway of tyrosine kinase receptors seems to require the Gq-coupled receptor 5-HT_{2B} for proper signals in mouse cardiomyocyte development and adult function. Clinically, in addition to the role of these receptors during embryogenesis, they may be important as determinants in migraine, hypertension, heart failure, neurodegenerative diseases and brain maturation disorders such as schizophrenia or autistic behaviors.

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Figure Legend

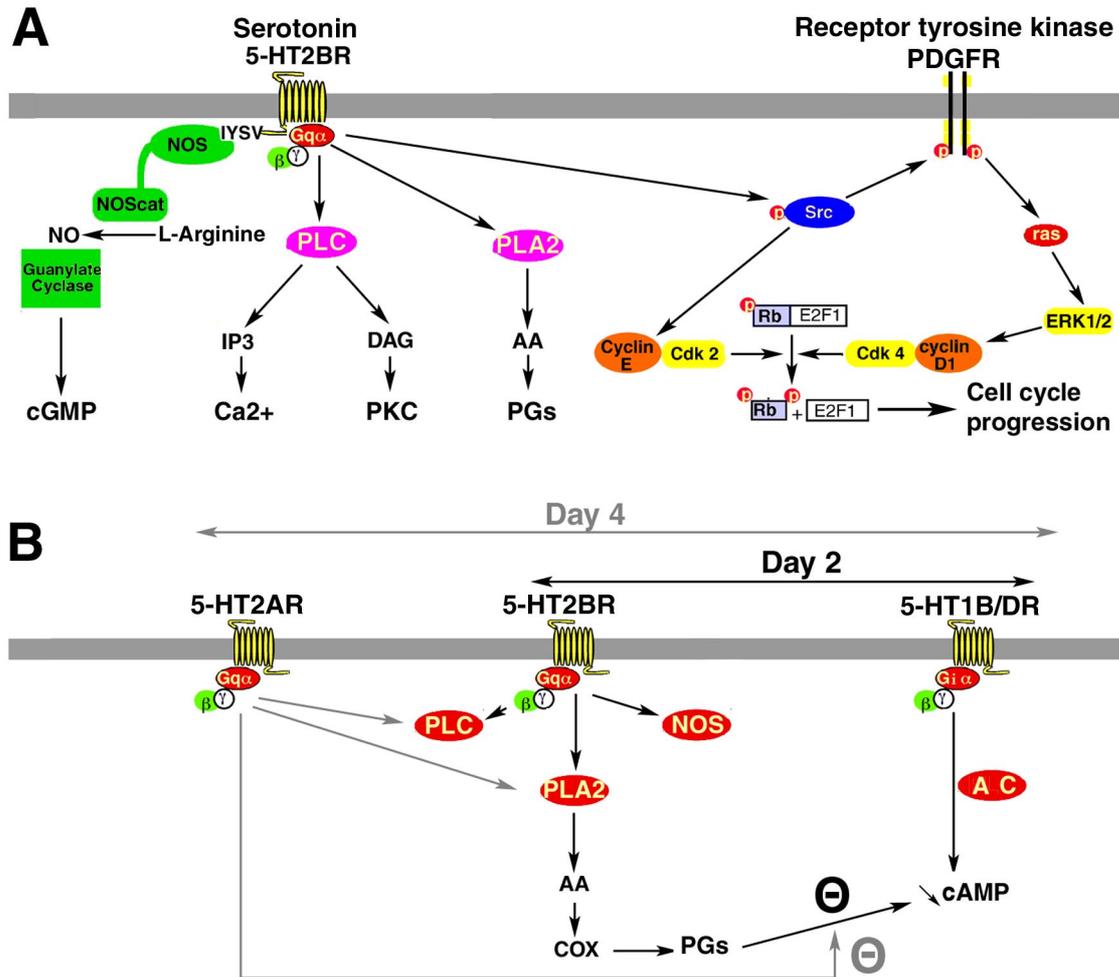


Figure 1 A. Signal transduction pathways of the 5-HT_{2B} receptor -Stimulation of the 5-HT_{2B} receptor triggers intracellular cGMP production through activation of nitric-oxide synthase (NOS). The type I PDZ motif (VSYI) at the C-terminus of the 5-HT_{2B} receptor is required for the NOS transduction pathways. The 5-HT_{2B} receptor couples with phospholipase C (PLC), to generate inositoltrisphosphate (IP₃) that releases intracellular calcium (Ca²⁺), and diacylglycerol (DAG) that activates protein kinase C (PKC), similar to the two other members (5-HT_{2A} and 5-HT_{2C}) of the 5-HT₂ receptor family. The 5-HT_{2B} receptor also activates the phospholipase A₂ (PLA₂) / arachidonic acid (AA) -dependent cyclooxygenase (COX) pathway. Activation of the 5-HT_{2B} receptor stimulates a ras-mitogen-activated protein kinase (ERK/MAPK) cascade. It appears that c-Src provides a link between PDGFR/MAPK and cell-cycle regulators in Gq-coupled 5-HT_{2B} receptor mitogenic signaling. c-Src either acts alone to control cyclin E induction or in concert with the PDGFR to induce cyclin D₁ expression via the ERK/MAPK pathway. This action raises the level of retinoblastoma (Rb) phosphorylation and triggers cell-cycle progression. B. Role of the 5-HT_{2B} receptors in *in vitro* differentiation. In the 1C11 serotonergic cell line, 5-HT_{1B/D} receptor function is controlled by 5-HT_{2B} and 5-HT_{2A} receptors through their cross-talk via the AA pathway. At day 2 of differentiation, activation of 5-HT_{2B} receptors, via a PLA₂/AA-dependent COX pathway, reduces the inhibition of cAMP induced by the 5-HT_{1B/D} receptor. At day 4 of differentiation, the PLA₂ can be stimulated by both 5-HT_{2B} and 5-HT_{2A} receptors. However, the 5-HT_{2B} receptor is not able to affect the 5-HT_{1B/D} receptor-dependent cAMP inhibition when the 5-HT_{2A} receptor appears. AC, adenylyl cyclase; PGs, prostaglandins.

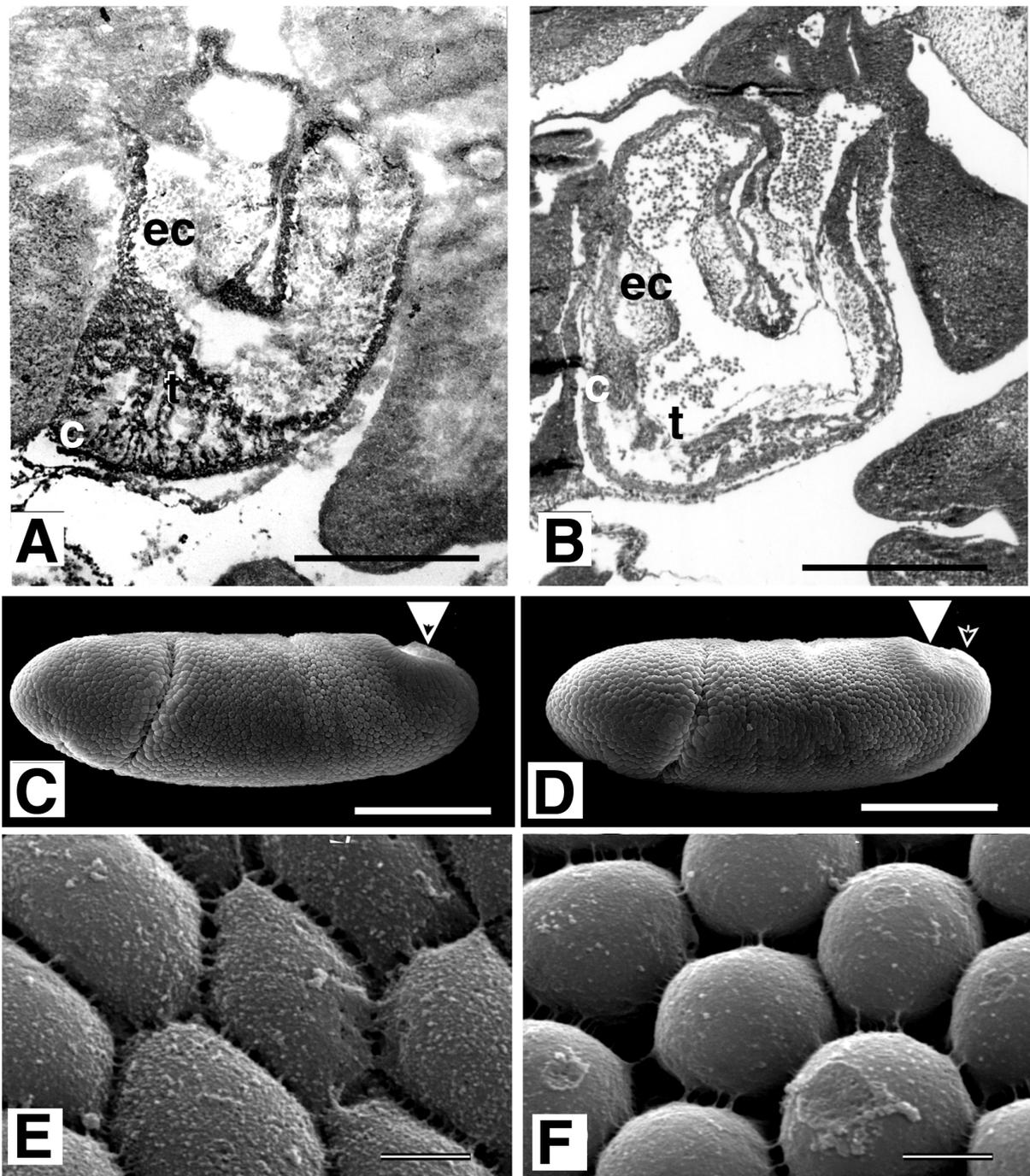


Figure 2-A-B. Sections of the heart region of a 10.5 day post coitum wild-type embryo stained with anti-5-HT_{2B} antibody (A) and of 5-HT_{2B} mutant embryo (B). These sections demonstrate a severe reduction in the thickness of the ventricle including both the compact zone (c) and the trabeculae (t) in 5-HT_{2B} -mutant embryos. (ec) endocardial cushions. C-F. 5-HT_{2Dro}-dependent modifications of cellular junctions during germband extension. The morphology of ectodermal epithelium observed in scanning electron microscopy for (C-E) control embryos appears abnormal in (D-F) 5-HT_{2Dro} deficient genotyped embryos. The shape of ectodermal cells is hexagonal with numerous microvilli in control (E) but round in mutant (F). The dorso-lateral course of proctodeal invagination (white arrowhead) and of pole cells (small black arrowhead) is indicated. Scale bars (A, B) 100 μm, (C, D) 150 μm, and (E, F) 2 μm.

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