Disturbed development of the enteric nervous system after \textit{in utero} exposure of selective serotonin re-uptake inhibitors and tricyclic antidepressants. Part 1: Literature review

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The increase in selective serotonin re-uptake inhibitor (SSRI) use during pregnancy, questions concerning abnormal development of the enteric nervous system (ENS), increase in laxative use in children and the association of fluoxetine with infantile hypertrophic pyloric stenosis (IHPS) gave rise to this pharmacological literature review. The role of 5-HT and the NE uptake in ontogeny of the ENS and the effects SSRIs and TCAs might have on the development of the ENS were investigated. The literature study showed that SSRIs may influence the development of the ENS in two ways. Blockage of the serotonin re-uptake transporter (SERT) during foetal development could influence migration, differentiation and survival of cells. This could lead to abnormal development in the first trimester of pregnancy. The other way is that 5-HT seems to be a growth factor in the primitive ENS. This growth factor like action is mediated through the 5-HT2B receptor and stimulation of this receptor by SSRIs influences the fate of late-developing enteric neurons. This could lead to abnormal development in the second and third trimester. TCAs could influence the development of the ENS, besides through inhibition of the SERT, through inhibition of the norepinephrine transporter (NET). Expression of the NET seems to be essential for a full development of enteric neurons and especially for serotonergic neurons. In addition the NET was detected early in ontogeny and precedes neuronal differentiation, which suggests that TCAs might influence development of the ENS when exposed early in pregnancy. The insights of this study gave rise to hypotheses which will be tested in an epidemiological cohort study.

Introduction

The prevalence of women suffering depression during pregnancy is estimated at 5–15% [1]. Selective serotonin re-uptake inhibitors (SSRIs) are the first line pharmacotherapy for depression and the most frequently used antidepressant in pregnant women. Approximately 2% of all pregnant women use antidepressants [2, 3] and the use of SSRIs has increased two- to four-fold in the western population during the last decade [2, 4]. SSRIs cross the placenta [5, 6] and concerns have been raised about their safety in pregnancy. The use of SSRIs in early pregnancy is associated with congenital heart defects [7] and use in late pregnancy has been associated with peripheral pulmonary hypertension [8]. There is a two-fold increased risk of congenital malformations overall [9–11]. Maternal use of SSRIs has been associated with cardiac malformation in some [7, 9, 12–16], but not all studies [17, 18] and with the risk of omphalocele, anencephaly and craniosynostosis [18]. Following these reports, the use of SSRIs during pregnancy is controversial [1]. It is however possible that not all SSRIs have the same teratogenic effects, since they differ in...
pharmacodynamic and pharmacokinetic properties [19]. Paroxetine has been associated with a 1.7-fold increased risk of cardiac malformations [14], but only few studies have assessed the risk of malformation associated with the use of specific SSRIs and their results are inconsistent [7, 12, 13, 15, 16, 18].

In addition Bakker et al. reported an association between maternal use of fluoxetine and infantile hypertrophic pyloric stenosis (IHPS) [20]. This relation was observed in a systematic surveillance study in which they investigated the occurrence of specific birth defects in relation to first trimester use of specific SSRIs. In Eurocat Northern Netherlands (population-based birth defects registry) they found in total three (1.7%) children with IHPS who were exposed to fluoxetine compared with eight fluoxetine exposures with other malformations (0.2%). The biological explanation includes the role of serotonin (5-HT) in the motility of the gastrointestinal tract (GI tract), the fact that fluoxetine passes the placenta and that 5-HT might play a role in the development of late developing enteric neurons. However, the exact mechanism of IHPS caused by fluoxetine exposure during pregnancy is still not fully elucidated.

Subsequent to the increased use of SSRIs in pregnancy and to the report that there might be a relation between maternal SSRI use and IHPS, an increase in laxative use in children was observed. These findings suggest the possibility of an association between the use of SSRIs during pregnancy and abnormal function of the GI tract in children. Questions have been raised concerning abnormal development of the GI tract and in particular the enteric nervous system (ENS) by scientists who were investigating the ontogeny of the ENS [21, 22]. However researchers never looked into the mechanism of SSRIs and the effect they may have on the foetal development of the ENS specifically. The observation of Bakker et al. is the first epidemiologic study in which an association between maternal SSRI use and abnormal function of the GI tract in children has been documented [20]. The gathering of these assumptions led to this set-up of a pharmacologic literature study and epidemiologic cohort study concerning the development of the ENS when exposed to SSRIs. In addition, leads concerning tricyclic antidepressant (TCA) exposure and the developing ENS were found [23, 24]. It may be possible that not only inhibition of the 5-HT transporter (SERT) but also of the norepinephrine transporter (NET) interferes with the growth of neurons in the developing ENS [23, 24]. Exposure to TCAs was therefore included in these studies. Congenital neuromuscular disorders of the bowel are both common and serious [25]. Confirmation by definitive epidemiologic studies would contribute to the theory that 5-HT and norepinephrine (NE) signalling pathways are important in embryonic development of the gut.

A literature study was performed on the role of 5-HT and NE uptake in ontogeny of the ENS and the effects SSRIs and TCAs might have on the development of the ENS. These results will be used to set up a cohort study in which use of laxatives and anti-diarrhoeal medication in childhood after SSRI and TCA exposure in utero will be investigated. Laxatives and anti-diarrhoeal medication use will be applied as a proxy for, respectively, constipation and diarrhoea, which could indicate disturbed development of the ENS.

**Literature search strategy**

A systematic search approach was used to find literature in PubMed about the influence of SSRIs on the development of the ENS. Several search strategies were used in the different chapters. The MeSH words that were used were based on the leads that were found during the literature study. First we looked into the embryology and ontogeny of the ENS (MeSH terms: enteric nervous system development/ontogeny). Since SSRIs influence the serotonergic neurons and 5-HT concentrations the articles that did not contain information about serotonergic neurons or 5-HT were excluded. Since Bakker et al. [20] reported an association between the use of SSRIs and IHPS we looked into the pathogenesis to see if the ENS contributed to this defect (MeSH terms: infantile hypertrophic pyloric stenosis pathiology). The most recent reviews were used. Due to the leads found in these articles we looked into a specific variable that is associated with IHPS: lack of interstitial cells of Cajal (ICC) in patients with IHPS (MeSH terms: infantile hypertrophic pyloric stenosis; MeSH terms: interstitial cells of Cajal). We excluded all articles that did not contain information about the role of 5-HT and the ICC. The findings so far led us to believe that a certain 5-HT receptor was mainly involved in the development of the ENS and gastrointestinal tract (GI tract). We searched specifically for the development of the ENS and the 5-HT<sub>2B</sub> receptor (MeSH terms: 5-HT<sub>2B</sub> development enteric nervous system; MeSH words: interstitial cells of Cajal, 5-HT<sub>2B</sub>). The association between IHPS and SSRIs was only found for fluoxetine. From a pharmacologic point of view it is important to know the affinity of fluoxetine for the 5-HT<sub>2B</sub> receptor (MeSH terms: fluoxetine, 5-HT<sub>2B</sub>). In addition the effects of TCAs on the NET were investigated and NET’s role in foetal development (MeSH terms: norepinephrine transporter, enteric nervous system).

The drug and molecular target nomenclature conforms to BJ's Guide to Receptors and Channels [26].

**The ontogeny of the ENS**

The ENS is the intrinsic innervation of the gastrointestinal tract and consists of neurons and glial cells that are phenotypically diverse [27]. These neurons and glial cells are grouped within two plexi, the myenteric (Auerbach’s) plexus, and the submucosal (Meissner’s) plexus [28]. Most
of the neurotransmitters that are expressed in the central nervous system (CNS) are represented within subclasses of the phenotypically diverse neurons of the ENS [29]. The functions of the ENS are to regulate the motility in the gut, to coordinate processes such as secretion and absorption, control of blood flow and the modulation of immune and endocrine functions [28]. The ENS is part of the peripheral nervous system (PNS) and shows independence from the CNS. Unlike all the other parts of the PNS, the ENS is capable of manifesting neurally mediated reflex activity in the absence of input from the CNS [25, 28]. Because of this special nature of the ENS there must be something that happens in the embryonic development that sets the ENS apart from the other divisions of the PNS. The cells from the ENS are, like the majority of the peripheral neurons and Schwann cells, descendants of colonizing neural crest cells (NCC) [25, 27, 30]. On the nineteenth day the neural plate appears and in the course of the third week the edges of the neural plate rise up and become neural folds, enclosing the neural groove. After the twenty-fifth day the neural folds merge to form the neural tube. While the neural tube is closing, cells on the lateral side of the neural plate detach themselves and form the neural crest. The NCC are distinguished by their great migrating ability and phenotypic heterogeneity, since very diverse cell types will arise from them [31] (Figures 1, 2).

There are three axial levels of the crest that have been shown to contribute to the ENS. These include the vagal, rostral-truncal and sacral levels [25]. The ENS is mainly derived from vagal NCC, adjacent to somites 1–7. The NCC from the vagal region populate the entire length of the gut, whereas the sacral NCC contributes to the hindgut [27, 28]. Vagal NCC migrate away from the neural tube towards the foregut [28]. The entire gut is colonized with vagal NCC by week 7 of development [27, 28, 30]. By this time the hindgut is not yet colonized by the sacral NCC. The cells derived from the sacral region of the neural crest colonize the hindgut after it has been colonized by vagal NCC. Sacral NCC first accumulate in the hindgut and form the nerve of Remak. Here they reside until they migrate into the hindgut along these nerve fibres and colonize there [27].

So the ENS is derived from cells that originate from two specific axial levels of the neural crest, the vagal and sacral neural crest. The formation of the ENS by these cells requires a co-ordinated migration, proliferation, differentiation and survival of NCC within the developing gut. Specific molecules in the wall of the gut influence the development of the enteric neurons, but also numerous genes and signalling molecules. In order to develop normally, the migrating NCC interact with signalling cues that are encountered along their migration pathways [30]. Interactions between migrating NCC and the local gut environment are essential for normal ENS development. A growth factor that is globally required for the development of all enteric neurons and glia is glial cell line-derived neurotrophic factor (GDNF) [25]. The receptor component (RET) is expressed on migrating NCC and GDNF is expressed in the gut mesenchyme [30]. The stimulation by GDNF is an absolute requirement for the survival of the vagal crest cells that colonize the gut [25]. Both vagal and sacral crest-derived cells are RET-dependent. The proneural gene mash-1 seems to be a requirement as well. A mutation of the mash-1 gene causes the oesophagus to become aganglionic and leads to loss of about one-third of the neurons in the remainder of the bowel. RET-dependent cells fail to develop in mash-1 (−/−) animals. These RET-dependent cells consist of a set of neural precursors, which are all transiently catecholaminergic before they acquire their terminally differentiated phenotype. These cells leave

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**Figure 1**
The forming of the neural crest in which the neural groove forms. The cells of the future neural crest are in orange. The arrows show the direction of the lateral folding. (A) Neural plate stage; (B) Neural groove stage; (1) epiblast; (2) neural groove; (3) neural crest. Picture courtesy of the universities of Fribourg, Lausanne and Bern (Switzerland)

**Figure 2**
The formation of the neural groove is followed by the formation of the neural tube. Cells detach themselves from the lateral side of the neural plate and form the neural crest. As soon as the NCC leave the neuroepithelium they lose their cohesion. (A) Migrating NCC (neural groove stage); (B) Neural crest after a completed detachment (neural tube stage); (1) epiblast; (2) neural fold; (3) migrating NCC; (4) neuroepithelium; (5) central canal; (6) neural tube. Picture courtesy of the universities of Fribourg, Lausanne and Bern (Switzerland)
the cell cycle to differentiate into early developed enteric neurons [25, 32]. This includes all serotonergic neurons of the gut (which are also missing in mash-1 (−/−) mice). The neurons that are mash-1 independent are never catecholaminergic and develop late in ontogeny. All enteric neurons that contain calcitonin gene-related peptide are members of this set. The transiently catecholaminergic cells also accumulate NE [33], but the responsible transporter has not been defined. Possible transporters are the dopamine transporter, organic cation transporters and the NET [24].

The role of 5-HT in the ontogeny of the ENS

The gut contains over 95% of the body’s 5-HT and the concentration of 5-HT in the bowel is an order of magnitude higher than that in the brain [34]. Peripheral nerves that contain 5-HT have also been detected in the heart, lung, kidney, spleen, thyroid and in blood vessels. 5-HT activates the 5-HT receptor and at least 14 different subtypes of the 5-HT receptor have been identified. The activity of 5-HT on 5-HT receptors is limited by the 5-HT transporter (SERT). SERT accumulates 5-HT in blood platelets and the re-uptake of 5-HT in the gut prevents desensitization of 5-HT receptors affecting motility. These are functions of 5-HT in the adult, but there is a lot of evidence that suggests that 5-HT modulates cell migration, differentiation and survival through certain 5-HT receptors [21, 22, 25, 32, 34–38].

As stated above all serotonergic enteric neurons are mash-1 dependent and develop early. Enterochromaffin cells (EC), which are the largest source of 5-HT, also develop before mash-1 independent enteric neurons [25, 32]. The early derived serotonergic neurons are also found to coexist in primordial enteric ganglia with still-dividing neural precursors [25]. These findings led to the assumption that 5-HT is a growth factor that affects the development of late-arising enteric neurons in the primitive ENS [32, 39]. There is also evidence that the developmental action of 5-HT is mediated by the transient and developmentally regulated expression of 5-HT receptors. This specific receptor is highly expressed and developmentally regulated in primordial enteric ganglia [32]. The 5-HT expression follows that of sources of 5-HT and coincides with the period of terminal differentiation of mash-1-independent enteric neurons [32]. 5-HT and 5-HT receptor agonists also promote in vitro differentiation of enteric neurons, whereas the promotion of neuronal differentiation is blocked by 5-HT receptor antagonists. These observations combined show that there is a possibility that stimulation of 5-HT receptors by 5-HT influences the fate of late-developing enteric neurons [32]. In the adult intestine the amount of neurons that express the 5-HT receptor is quite low (<5% of all neurons). However in foetal mice at E14-E16 in every ganglion of the developing myenteric plexus mRNA was found that encoded for the 5-HT receptor [39]. At E18 the proportion of neurons expressing mRNA encoding the 5-HT receptor declined to the low adult level [39]. These observations also corroborate the theory that the 5-HT receptor mediates the growth factor-like action of 5-HT on developing enteric neurons [21]. In addition to the neuronal lineage, the 5-HT receptor was also found on intestinal crypts [32] where they can affect growth and differentiation [40]. 5-HT stimulates the self-renewing stem cell population of the crypts of the gut [41, 42]. It is possible that the 5-HT receptor is linked to cell growth and differentiation in both neuroectodermal and endodermal derivatives of the gut. New neurons continue to be added to the ENS for at least 3 weeks postnatal in mice [43]. It is hard to determine the equivalent period in humans, but the differences in mice and humans suggest that new neurons are added to the postnatal human gut for much more than 3 weeks [32].

Not only 5-HT receptors might play a role in the development of the ENS. The SERT may also affect the migration and differentiation by regulating the 5-HT concentrations in the NCC environment. SERT mRNA is expressed throughout the developing embryo beginning prior to organogenesis [22]. Expression of SERT follows sensory pathways and this suggests that 5-HT may play a role in setting up patterns of connectivity critical to processing sensory stimuli [22]. Mice that lack the SERT do not develop normally and survive to adulthood [21], but periods of diarrhoea and constipation are observed [44]. The diarrhoea in these mice is associated with excessive colonic motility which results in increased excretion of water in the stool [44]. During periods of constipation the motility is excessively slow. This can be explained by desensitization of 5-HT receptors. Mice that lack the SERT cannot handle the challenge of changing 5-HT concentrations [38]. This shows that SERT is important in the regulation of 5-HT but not as important as the 5-HT receptor in the development of enteric neurons.

The role of the norepinephrine transporter in the ontogeny of the ENS

As explained in ‘the ontogeny of the ENS’ the neural crest precursors of the ENS are transiently catecholaminergic while still proliferating. These cells also accumulate NE and there are indications that the NET might be the transporter. The NET is expressed on a subset of noncatecholaminergic enteric neurons and was detected in all regions of the mouse gut [24]. Enteric NET expression was detected at E10, which precedes neuronal differentiation [24], and peaked at E12 coincidentally with early neurogenesis [24]. This corresponds with respectively E21 and E41 in humans. NET expression during sympathetic neuronal development has been linked to acquisition of the noradrenergic phenotype. Ablation of the NET gene leads to deregulation of 5-HT signalling pathways that are involved in the ENS development and to changes in ENS function. These
changes in peristalsis may lead to lethal intestinal pseudo-obstruction and colonic hyperganglionosis [23].

In NET knock-out mice expression of the NET seems to be essential for a full development of enteric neurons and especially for serotonergic neurons. The ENS of the NET knock-out mice contained fewer neurons than that of wild-type littermates and especially the serotonergic neurons were effected [45, 46]. However, NET expression is not essential for the development/survival of all of the neurons that express it but absence particularly affects serotonergic neurons. It is yet unknown how these NET effects are mediated [24]. The known role of NET is to remove NE from the synaptic cleft [47, 48]. NE uptake from the synaptic cleft might not be the only function of NET. The developmental action could be independent from the removal of NE [24]. Li et al. suggest that the regulation of the intracellular NE concentration by NET could be important to its developmental effects. They speculate that since depolarization is known to facilitate enteric neuronal development [49] and since NET is electrogenic, NET may also enhance development by NE-induced ion transport [24]. A direct effect of NE on development of the ENS has not been described in the literature.

**Infantile pyloric stenosis and the development of interstitial cells of Cajal**

During the development of infantile hypertrophic pyloric stenosis (IPHS) the pylorus becomes enlarged so that the pyloric sphincter is unable to open. This causes a distal obstruction of the GI tract and this obstruction prohibits stomach contents to pass through the entire GI tract which results in emesis [50, 51]. IHPS is the most common surgical cause of vomiting infants [51]. The occurrence of IHPS has been associated with several variables such as genetic, environmental and mechanical factors. The function and the motility of the pyloric sphincter are controlled by a complex system which involves the ENS, gastrointestinal hormones and ICC.

The ICC are nonneuronal cells that form networks alongside the ENS and contribute to normal motility in the GI tract. They serve as the electrical pacemaker currents, also known as slow waves and are essential for effective peristalsis. The myenteric ICC trigger these spontaneous pacemaker currents and the intramuscular ICC mediate excitatory and inhibitory neurotransmission [37, 51]. The ICC are a minor component of the tunica muscularis of the GI tract, but they are considered to be the most important cells that are co-ordinating gastrointestinal motility. ICC are found to be absent or reduced in numbers in patients with IPHS when compared with controls [51, 52]. This might explain the gap in slow waves of peristalsis and may be responsible for the altered state of the pyloric sphincter.

The 5-HT_{2B} receptor is one of the four 5-HT receptors (5-HT_{1A}, 5-HT_{1D}, 5-HT_{2B} and 5-HT_{2C}) that are known to regulate proliferation and survival of cells. In the GI tract, only the 5-HT_{2B} has been linked to the regulation of enteric neuron survival and ICC proliferation [36, 37]. The 5-HT_{2B} receptor is expressed on ICC in adult mice and the activation of these receptors increases proliferation of ICC cultured from neonatal mouse jejunum [37]. Tharayil et al. [36] showed that 5-HT_{2B} signalling is important in the balance of factors that regulate ICC network volume and ICC numbers. The lack of 5-HT_{2B} receptors (by using knock-out mice) reduced the volume of ICC by 30% in the myenteric plexus region and 40% in the deep muscular plexus region. The number of ICC was also reduced.

**Mechanism of action of SSRIs and TCAs**

SSRIs and TCAs are commonly prescribed drugs in major depressive disorder (MDD). Their working mechanisms, related to MDD, are based on the monoamine hypothesis. This theory states that depression is caused by a deficiency of monoamine transmitters at certain areas in the brain [53]. Initially the hypothesis was formed in terms of NE, but subsequent work showed that most of the observations were equally consistent with 5-HT and dopamine (DA) being the key mediators [53, 54]. All antidepressants increase the neurotransmission of NE, 5-HT and/or DA. The key to their action is the desensitization of certain neurotransmitter receptors. The increase in neurotransmission by antidepressants causes the desensitization of the receptors which translates to an antidepressant effect [54]. There are numerous theories about the antidepressant effect, but the interpretation is limited by the lack of a complete theory of the development of mood disorder. However for some antidepressants it is known to which receptors they bind and what their affinity is [19], but what the effect on MDD is remains not fully understood.

When a SSRI is administered it binds directly to the SERT. SERT terminates the action of 5-HT by transporting 5-HT from the synaptic spaces into presynaptic neurons. Through the inhibition of the SERT the 5-HT concentration in the synaptic cleft is increased [54]. SSRIs can also bind to 5-HT receptors [55, 56]. Fluoxetine appears to be an agonist at 5-HT_{2B} receptors, but is an antagonist at the 5-HT_{2C} receptor on astrocytes [35, 57]. It is not unusual for 5-HT ligands to act differently on 5-HT_{2B} and 5-HT_{2C} receptors [57, 58]. Fluoxetine has a non-negligible affinity for the 5-HT_{2C} receptor, which paroxetine does not share. However paroxetine has a higher affinity for the SERT than fluoxetine [19]. Similar 5-HT_{2B} receptor-mediated acute effects in cultured astrocytes as for fluoxetine were found with paroxetine [59]. It is suggested that 5-HT_{2B} Receptors are likely to be activated during chronic treatment with an SSRI [59]. Chronic use of fluoxetine somehow initially down-
regulates and subsequently up-regulates 5-HT_{2B} effects in astrocytes during a treatment period similar to that required for fluoxetine’s clinical response to occur [35]. Chronic therapeutic concentrations of SSRIs may lead to binding to 5-HT_{2B} receptor sites in other organs: pulmonary hypertension appears to be correlated with increased 5-HT_{2B} receptor activation [60, 61] and persistent hypertension in the new born shows a statistically significant increase of three to four times when exposed to SSRIs in utero [62]. Metabolites of SSRIs can also contribute to the therapeutic effect. Fluoxetine, for example, is a strong inhibitor of the SERT but also has a weak affinity for the NET. Its metabolite, N-desmethylfluoxetine, is a strong inhibitor of the SERT as well, but more selective than fluoxetine.

The mechanism of action of TCAs in depression is due to re-uptake inhibition of 5-HT and NE. Most TCAs have an affinity for the NET [19]. The NET is a Na\(^+\) and Cl\(^-\) dependent transporter and drugs that block NET, like TCAs, inhibit the NE transport. The serotonergic action of TCAs works through binding to the SERT but other 5-HT receptors might also be involved. Selectivity of inhibition of the SERTs vs. NETs differs across the family of the TCAs [54]. The inhibition constant \(K_i\) is a measure of the binding affinity of a ligand to its receptor. \(K_i\) is the concentration of the ligand at which 50% of the receptors are occupied by the ligand. While most metabolites of TCAs also bind to some receptors, it is evident that they also contribute to the therapeutic effect [19]. Amitriptyline is a strong inhibitor of the SERT and the NET (SERT: \(K_i = 22.71\) nM; NET: \(K_i = 46.46\) nM). The metabolite of amitriptyline is nortriptyline and is a strong inhibitor of the NET (SERT: \(K_i = 129.40\) nM; NET: \(K_i = 7.39\) nM). Clomipramine is a strong inhibitor of the SERT and is the most selective TCA (SERT: \(K_i = 0.21\) nM; NET: \(K_i = 45.85\) nM). If these \(K_i\) values are compared with those of paroxetine (SERT: \(K_i = 0.29\) nM; NET: \(K_i = 130.80\) nM) and fluoxetine (SERT: \(K_i = 5.92\) nM; NET: \(K_i = 600.0\) nM), then they are in the same order of magnitude. The characteristics of clomipramine alone are similar to those of SSRIs, but its metabolite N-desmethylclomipramine is a more potent and selective inhibitor of the NET [19]. The half-life of N-desmethylclomipramine (36 h) is long compared with clomipramine (21 h) [19]. The concentration ratio of clomipramine and N-desmethylclomipramine in maternal blood is approximately 1 [63] so the metabolite contributes greatly to the therapeutic effect because of the noradrenergic effects and the longer half-life. Clomipramine itself can be considered to have the same characteristics as SSRIs, but due to the actions of its metabolite and chemical structure it is classified as a TCA (Table 1).

The effects of antidepressants on the development of the ENS

A relationship has only been found between IHPS and fluoxetine and not with any other SSRI or TCA [20]. This suggests that fluoxetine has a characteristic that no other SSRI

| Receptor | Drug [19, 59] | Action on receptor | Neurotransmitter role | Effects |
|----------|---------------|-------------------|----------------------|---------|
| 5-HT_{2B} | Fluoxetine Paroxetine | Agonist of 5-HT_{2B} on astrocytes and initially down-regulates and subsequently up-regulates 5-HT_{2B} effects (astrocytes) Causes desensitization and/or down-regulation of 5-HT_{2B} in the fundus | 5-HT seems to be a growth factor in the primitive ENS and acts through the 5-HT_{2B} receptor | Effects the development of late-arising neurons (second and third trimester) |
| SERT | Amitriptyline Clomipramine Paroxetine Fluoxetine Sertraline Fluvoxamine Escitalopram Citalopram Imipramine Desipramine | SERT inhibitor | Concentrations of 5-HT change because the SERT is blocked | Influences migration, differentiation and survival of cells (mainly first trimester) |
| NET | Amitriptyline Nortriptyline N-desmethylclomipramine Imipramine Desipramine | NET inhibitor | Regulation of the intracellular NE concentration by NET could be important to its developmental effects | NET seems to be essential for a full development of enteric neurons and especially for serotonergic neurons. NET expression occurs early in ontogeny (first trimester) |

The developing ENS and antidepressant exposure in utero: Part 1

Table 1
Summary of the main mechanisms by which SSRIs and TCAs affect the development of the ENS

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or TCA shares. Bakker et al. [20] suggested that the affinity of fluoxetine for 5-HT2C might play a role. Fluoxetine has the highest affinity for the 5-HT2C receptor compared with other SSRIs [19, 64]. However when the affinity of fluoxetine for the 5-HT2C receptor is compared with TCAs the affinity is much lower. Where at therapeutic concentrations the receptor occupancy of fluoxetine is 19.74% for the 5-HT2C receptor, the occupancy of TCAs, such as amitriptyline and doxepin, is higher than 90% [19]. There was no association found between TCAs and IHPS in epidemiologic studies so this rules out the 5-HT2C receptor.

The SSRIs sertraline, fluvoxamine, escitalopram, paroxetine, fluoxetine and citalopram show a very high affinity for the SERT compared with the other antidepressants [19]. Since SERT plays a role in the development of the ENS by regulating the 5-HT concentrations, blockage of these transporters during foetal development could influence migration, differentiation and survival of cells.

Several studies suggest that the 5-HT2B receptor plays a primary role in the development of the ENS [21, 25, 32, 35–37]. It is known that fluoxetine has an agonistic action at 5-HT2B receptors. As stated above the chronic use of fluoxetine initially down-regulates and subsequently up-regulates 5-HT2B effects in astrocytes. It is unknown what effect fluoxetine has on the 5-HT2B receptor in the gut. Usually 5-HT2B agonists induce desensitization and/or down-regulation of the receptors in the CNS. This has been reported for the 5-HT2A and 5-HT2C receptor [65–73]. The peripheral 5-HT2B receptor has a high degree of homology with the central 5-HT2C receptor. The 5-HT2C and 5-HT2B receptors give rise to a more rapid loss of function than 5-HT2A receptors [74]. This all suggests that the 5-HT2B receptor is desensitized and/or down-regulated by agonists. These experiments are performed with peripheral 5-HT2B receptors (from the fundus), and not with central 5-HT2B receptors (astrocytes). It is most likely that the receptors in the ENS will respond similarly to the 5-HT2B receptors from the fundus. Down-regulation of 5-HT2B receptors changes the proliferation of ICC which are essential for effective peristalsis [75, 76]. The lack of 5-HT2B receptor signalling reduces the density of ICC network which results in a gap in slow waves of peristalsis [36].

SSRIs that bind to the 5-HT2B receptor might influence the development of the ENS. Since there is evidence that certain SSRIs, such as fluoxetine, but also paroxetine, have affinity for this receptor the use of these compounds during pregnancy requires extreme care. Fluoxetine (5-HT2B: \( K_i = 5300 \, \text{nm} \)) [77] shows a higher selectivity for the 5-HT2B receptor than paroxetine (5-HT2B: \( K_i = >10,000 \, \text{nm} \)) [78]. The selectivity for receptor inhibition is high enough that fluoxetine given therapeutically may activate (astrocystic) 5-HT2B receptors [59]. If fluoxetine or paroxetine binds to the 5-HT2B receptor, 5-HT cannot activate these receptors to stimulate the development of the ENS. Thus changes in 5-HT concentrations caused by SSRIs and the binding affinity of specific SSRIs, such as fluoxetine and paroxetine, to the 5-HT2B receptor may contribute to a disturbed development of the ENS. So far there is no evidence that the use of SSRIs consistently results in major malformation or obvious congenital defects. There are studies that have found an association between maternal clomipramine use and ventricular or atrial septal defects [79] (838 clomipramine exposures) and other studies have found no teratogenic effects of TCA use during pregnancy [80–83] (TCA exposure ranges from 45 cases [83] to 438 cases [82]). It may lead to less obvious defects such as disturbed function of the GI tract as are reflected in overuse of laxatives and anti-diarrhoeal medication due to periods of constipation and diarrhoea. There also may be critical periods of vulnerability when foetal exposure to SSRIs might affect certain systems more than another [22]. It is expected that SSRI use during the first trimester causes the most defects. At week 7 of the pregnancy the gut is colonized by vagal NCC and approximately 1 week later the first serotonergic neurons derive [25, 27]. Migration of NCC to the gut is very important for normal development. However addition of enteric neuron persists for a long time, even postnatally. So during the whole pregnancy there is a chance of disturbance in the development of the ENS when exposed to SSRIs, but mainly during the first trimester. It should be mentioned that SSRIs also influence vascular endothelial growth factor (VEGF) in the CNS [84] and at a systemic level [85]. Since VEGF plays an important role in embryogenesis, neurogenesis and angiogenesis, this mechanism could contribute to the effects SSRIs have on the development of the ENS.

If we compare the mechanism of action of TCAs with SSRIs it is surprising that no relationship between IHPS and TCAs was found. The selectivity of inhibition of the SERT vs. the NET differs across the family of TCAs [54]. Amitriptyline, clomipramine and imipramine show a high affinity for the SERT like some SSRIs (for \( K_i \) values see ‘Mechanism of action of SSRIs and TCAs’) [19]. Blockage of the SERT during foetal development could influence the development of the ENS through a serotonergic pathway. However, the metabolites can have a different selectivity ratio for the SERT and the NET. If the selectivity for the transporter changes from SERT to NET the effect on the development of the ENS may change. Clomipramine was used as an example. Clomipramine is a strong inhibitor of the SER and N-desmethylclomipramine is a more potent and selective inhibitor of the NET. The half-life of the metabolite also plays an important role in the therapeutic effect since the concentration ratio of clomipramine and N-desmethylclomipramine in maternal blood is approximately 1 and the concentration ratio in umbilical cord blood is 0.66 [63]. In the foetus N-desmethylclomipramine is measured in a higher concentration and will have the upper hand in the therapeutic effect (ter Horst and others, submitted data). Since N-desmethylclomipramine has a higher affinity for the NET, clomipramine exposure will most likely affect the noradrenergic pathway of development.
also applicable for amitriptyline. The metabolite of amitriptyline is nortriptyline which is a preferentially strong inhibitor of the NET [19]. Both amitriptyline and nortriptyline cross the placenta but the transfer of nortriptyline is approximately 20% lower compared with amitriptyline [86]. So in addition to influencing the development of serotoninergic neurons these TCAs also influence the noradrenergic pathway. The expression of the NET seems to be essential for the full development of enteric neurons, specifically serotoninergic neurons. TCAs inhibit the differentiation of NCC into noradrenergic neuroblasts through the NET [23]. The enteric NET was found at E10 in mice and peaked at E14. This precedes neuronal differentiation, but also 5-HT_{2A} expression [24]. This suggests that blockage of the NET during this period with TCAs may lead to changes in function of the ENS. NET expression is important during this period and in utero exposure of TCAs during the first trimester will most likely interfere with normal ENS development. The fact that NET expression also precedes 5-HT_{2A} expression might suggest that exposure to TCAs during the first trimester may cause a more distinct effect on ENS development than SSRI exposure in the first trimester. No accounts were found that the NET influences neuronal development further in ontogeny.

No study or case report was found reporting a disturbed function of the GI tract when exposed to SSRIs or TCAs during pregnancy. This could be due to the perception that abnormal bowel function in children is not associated with in utero drug exposure and that it is not considered a severe abnormality.

These insights gave rise to an epidemiological study to test the following two hypotheses: (i) in utero exposure to SSRIs in the first trimester, but also the second and the third trimester will lead to disturbed bowel function and (ii) in utero exposure to TCAs in especially the first trimester will lead to disturbed bowel function.

## Competing Interests

There are no competing interests to declare.

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