Chapter 4

Relation between platelet serotonin and feeding mode in newborns suggests that gut motor activity is a determinant of platelet serotonin content

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Kemperman RFJ, Bruins S, te Lintelo JTV, van der Dijs FPL, Erwich JJHM, Muskiet FD, Landman H, Kema IP, Muskiet FAJ. Relation between platelet serotonin and feeding mode in newborns suggests that gut motor activity is a determinant of platelet serotonin content. accepted for publication in Biogenic Amines
Abstract

*Background and Aims:* The usefulness of platelet serotonin (PLT 5-HT) as a marker of gastrointestinal motility is as yet unclear. We determined whether PLT 5-HT is lower at a condition of relative gut motor activity quiescence (i.e. in newborns at birth) compared with a condition of normal gut motor activity (i.e. in their mothers at birth), and whether in newborns institution and discontinuation of enteral feeding coincide with increases and decreases of PLT 5-HT, respectively. *Design and Measures:* PLT 5-HT was determined in 17 mothers and their 18 healthy full term newborns. Longitudinal PLT 5-HT data and data of feeding modes were available for 5 preterm born infants. *Results:* Newborns exhibited about 2 times lower PLT 5-HT compared with their mothers (medians: 1.5 and 2.9 nmol/10^9 PLT, respectively). Newborn PLT 5-HT related positively with maternal PLT 5-HT and newborn mean PLT volume, and negatively with newborn whole blood tryptophan. In the longitudinally investigated preterm born infants we observed 7 increases and 1 decrease of PLT 5-HT during institution of enteral feeding (in 5 infants), and 2 decreases and 1 increase of PLT 5-HT during parenteral feeding (in 3 infants). *Conclusion:* Lower PLT 5-HT at birth and its change in response to enteral feeding in newborns suggest gut motor activity to be a determinant of PLT 5-HT in early postnatal life.
1. Introduction

Serotonin (5-hydroxytryptamine; 5-HT) is a biogenic amine that derives from the essential amino acid tryptophan (Tryp) by hydroxylation and subsequent decarboxylation. About 80% of the bodily 5-HT is localized in the enterochromaffin cells (90-95%) and neurons (5-10%) [1;2] of the gastrointestinal (GI) tract. Serotonin localized in the basolateral stores of enterochromaffin tissue is released upon neuronal, chemical or mechanical stimulation. Several 5-HT receptors control GI motility, sensation and secretion [1;2]. Following its release 5-HT is removed from the interstitial space by 5-HT selective reuptake transporters [3;4]. Part of the 5-HT also enters the portal blood and systemic circulation where it is either rapidly taken up and accumulated by platelets (PLT), or metabolized by the liver, lung and kidneys into its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) [2;5;6]. Platelets store and transport the majority (99%) of circulating 5-HT [7].

Gastrointestinal motor activity might be reflected by PLT 5-HT. Although not consistently found [8], patients with diarrhea predominant irritable bowel syndrome (d-IBS) have higher PLT 5-HT as compared with healthy controls [2]. The augmented GI motility in these patients with d-IBS is likely to cause increased exposure of their circulating PLT to 5-HT [2]. Increased PLT 5-HT is also observed in patients with carcinoid tumors [9]. Carcinoid tumors derive from enterochromaffin cells and diarrhea is a frequent symptom [10]. Notably those carcinoid tumors originating from the midgut are characterized by high 5-HT production and measurement of PLT 5-HT is used as a sensitive marker for the early diagnosis and the subsequent follow-up of patients with carcinoid tumors [9]. Finally, 36-58% of patients with autism have been reported to exhibit increased PLT 5-HT [11]. Patients with autism have a higher prevalence of GI disturbances, but this observation is controversial [12]. A recent study shows a 5.1 times higher odds of congenital anomalies of the GI tract in children with autism [13].

The usefulness of PLT 5-HT as a marker of GI motility is as yet unclear: increased GI motility may cause increased PLT 5-HT, while decreased motility may relate to low PLT 5-HT. The fetus exhibits little GI motor activity prior to 30 weeks of gestation, but this increases steadily thereafter [14]. After 32 weeks of gestation nonmigrating motor activity patterns lengthen and decrease in occurrence, and migrating motor complexes begin to appear [15]. The use of gut motor patterns to assess gut maturity has thus been suggested [14]. There is substantial evidence that intestinal motor activity in newborns becomes stimulated by enteral nutrition [16]. Better maturation of motor function [17] and increased intestinal trophic responses [18] are observed in enterally fed preterm animals, as compared with parenteral
feeding. Consequently, the rapid doubling of PLT 5-HT levels after birth to near-adult levels during the first postnatal days [19] is likely to be caused by enteral feeding instituted maturation of gut motor activity. The aim of the present study was to contribute to the development of PLT 5-HT as a marker of GI motility by investigating whether PLT 5-HT is lower at an established physiological condition of relative gut motor quiescence (i.e. immediately after birth) and whether it subsequently changes as a function of feeding mode (i.e. enteral or parenteral). Therefore we compared PLT 5-HT of newborns with that of their mothers and monitored the course of PLT 5-HT in a small number of newborns that we were able to study during switches from enteral to parenteral feeding and vice versa.

2. Materials and Methods

2.1. Patients and Samples

For the comparison of PLT 5-HT from newborns and their mothers, venous blood samples of 17 mothers were collected together with venous umbilical cord blood samples of their 18 newborns (i.e. 16 singletons and 1 twin). Both samples were taken at delivery. Pregnant women were eligible to participate if they gave informed consent, had uncomplicated pregnancies, had uncomplicated deliveries at term, and if they did not the following: substances of abuse other than alcohol and tobacco, psychotropic medication and drugs affecting PLT 5-HT levels (e.g. selective 5-HT reuptake inhibitors). The participants were recruited from 28 consecutive expecting mothers visiting the Department of Obstetrics and Gynecology. Only newborns that were appropriate or large for gestational age according to Kloosterman [20] were included in the final evaluation.

For the longitudinal study of neonatal PLT 5-HT we collected venous blood of 20 consecutive preterm born infants (16 singletons and 2 twins) who were admitted to the neonatal intensive care unit (NICU). Additional blood samples (0.5 mL) were collected only when during their stay hematological indices were ordered by the pediatrician. Data on the course of pregnancy and delivery, anthropometrics, medication and diet, GI function (e.g. vomiting, diarrhea, bowel movements etc.) were gathered from the patient records. Only neonates of whom longitudinal samples and clinical data were available were included in the final evaluation. In all cases, enteral feeding consisted of (fortified) breast milk or Similac Special Care (Ross Products Division, Abbott Laboratories, Columbus, OH, USA), whereas parenteral feeding consisted of a 5-10% dextrose solution with amino acids (Vaminolact®); Pharmacia-Upjohn, Sweden), electrolytes, vitamins and trace elements prepared by the hospital pharmacy, and separately Intralipid 20% solution (Kabi-Vitrum, Sweden).
The studies were conducted at the Department of Obstetrics and Gynecology and the Department of Pediatrics of the St. Elisabeth Hospital in Curaçao (Netherlands Antilles). Informed consent was obtained from the mothers or both parents. The study protocols were in accordance with local ethical standards and the Helsinki declaration of 1964, as revised in 2004.

### 2.2. Analyses

Blood samples were collected in K₂-EDTA, immediately stored in melting ice and divided into two portions. One portion was used for the assay of hematological indices. The second was used for HPLC profiling of indoles in whole blood. A mixture of K₂EDTA and Na₂S₂O₅ (1:1) to a final concentration of about 10 g/L was added to prevent oxidation. Samples were subsequently stored at -80 ºC until transportation on dry ice to the University Medical Center in Groningen (The Netherlands). Trpy, 5-hydroxytryptophan (5-HTP) and 5-HT were measured with a single HPLC-fluorometric profiling method, using a previously published method [21]. PLT 5-HT was calculated by dividing the whole blood 5-HT concentration by the PLT count. The outcome was expressed in nmol/10⁹ PLT.

### 2.3. Data evaluation and statistics

All data were analyzed using the Statistical Product and Service Solutions package, version 11.5 (SPSS Inc. Chicago). Data for newborns and their mothers were tested for normality using the Shapiro-Wilk W test. Non-Gaussian distributed indices were transformed by \( \log \) transformation. Differences between indices of newborns and their mothers were assessed with the paired Student's t-test with Bonferroni correction, at \( \alpha = 0.0036 \). Relations between indices of newborns and their mothers were evaluated with Pearson or Spearman correlation, following correction for multiple correlations at \( \alpha = 0.0036 \). Indices exhibiting correlations with newborn PLT 5-HT or whole blood 5-HT at \( \alpha = 0.05 \) were further investigated by backward linear regression analysis for their ability to explain the observed variance. The final model was obtained after exclusion of insignificant indices.

A change of PLT 5-HT was considered significant if it exceeded the reference change value (RCV) at a 95% confidence level [22]. The employed RCV was derived from the median intra-individual biological variation as established from PLT 5-HT data of 18 apparently healthy adults [12 males, 6 females; median (range) age 35.0 (20.8-56.6) years], who were monitored during five consecutive days (unpublished observations). Their median intra-individual coefficients of variation (CVi) for PLT 5-HT and whole blood 5-HT amounted to 7.3 and 7.4%, respectively. These figures, together with a 5-HT inter-assay variation (CVa) of \( \leq 2.8\% \) [21], resulted in RCV for PLT 5-HT and whole blood 5-HT of 21.6 and 21.9%,
respectively. Changes of newborn PLT 5-HT exceeding the RCV were related to changes in feeding mode as occurring within the corresponding time intervals.

3. Results

3.1. Newborn and maternal indices and their interrelations

Table 1 shows for both newborns and their mothers some anthropometric data, together with hematological indices, whole blood concentrations of Tryp, 5-HTP and 5-HT, and PLT 5-HT.

| Table 1. Platelet indices, and indoles in whole blood and platelets of newborns and their mothers at delivery |
|---------------------------------------|---------------------------------|-----------------|-----------------|
| **Anthropometrics**                   | **Newborns**                    | **Mothers**     | **P-value**     |
| Gender (Male / Female)                | 9 / 9a                          | 17              |                 |
| Age                                   | 38.9 (37.3-41.9) weeksb          | 33.1 (18.3-39.0) years |                 |
| Weight (kg)                           | 3.430 (2.680-4.410)              | 84.0 (64.0-122.0)c |                 |
| AGA / LGA                             | 16 / 2                          |                 |                 |
| **Platelet indices**                  |                                 |                 |                 |
| PLT (⁎10⁹/L)                          | 278 (±70)                       | 243 (±54)       | 0.018d          |
| MPV (fL)                              | 8.0 (6.6-11.6)                  | 9.2 (7.2-13.6)  | 0.532d          |
| **Indole concentrations**             |                                 |                 |                 |
| Tryp (μmol/L)                         | 43.1 (±8.1)                     | 21.7 (±4.0)     | < 0.001         |
| 5-HTP (nmol/L)                        | 206 (17-301)                    | 17 (17-246)     | 0.023b          |
| 5-HT (nmol/L)                         | 388 (240-712)                   | 618 (420-1856)  | < 0.001d        |
| 5-HT (nmol/10⁹ PLT)                   | 1.5 (0.8-3.2)                   | 2.9 (1.9-6.4)   | < 0.001d        |

Data are medians (range) or means (±SD). P<0.0036 was considered significant. a Two girls were twins. AGA, appropriate for gestational age [20]; LGA, large for gestational age [20]; PLT, platelets; MPV, mean platelet volume; Tryp, tryptophan; 5-HTP, 5-hydroxy tryptophan; 5-HT, 5-hydroxytryptamine; M/F male/female. b gestational age at birth; c n = 16; d Student’s t-test performed on ‘log transformed data; e concentration in whole blood; f 5-HTP was below the 17 nmol/L [21] detection limit in 13 mothers; g 5-HTP was below the 17 nmol/L [21] detection limit in 8 newborns; h Wilcoxon Signed-Rank test.

In all samples 5-HIAA was below the detection limit of 61 nmol/L [21]. Compared with their mothers, newborns had significantly (p<0.0036) higher whole blood Tryp, but lower PLT 5-HT and whole blood 5-HT. The following correlations (Pearson coefficient; p-value) between the corresponding indices of newborns and their mothers were found to be significant (p<0.0036): RBC (r=0.757; p<0.001), Ht (r=0.726; p=0.001) and whole blood 5-HT (r=0.743; p<0.001).
The \( \log \) PLT 5-HT of the newborns was found to be related to: newborn PLT (\( r = -0.567; p = 0.014 \)), newborn \( \log \) MPV (\( r = 0.476; p = 0.046 \)), newborn whole blood 5-HT (\( r = 0.686; p = 0.002 \)), newborn Tryp (\( r = -0.479; p = 0.044 \)) and maternal \( \log \) PLT 5-HT (\( r = 0.549; p = 0.018 \)). Newborn whole blood 5-HT correlated with: newborn \( \log \) PLT 5-HT (\( r = 0.686; p = 0.002 \)), maternal \( \log \) whole blood 5-HT (\( r = 0.743; p < 0.001 \)) and maternal \( \log \) PLT 5-HT (\( r = 0.741; p < 0.001 \)). The \( \log \) PLT 5-HT of the mothers was found to be related to: newborn birth weight (\( -0.520; p = 0.027 \)), newborn whole blood 5-HT (\( 0.741; p < 0.001 \)), newborn \( \log \) PLT 5-HT (\( 0.549; p = 0.018 \)), and maternal \( \log \) whole blood 5-HT (\( 0.794; p < 0.001 \)). The \( \log \) whole blood 5-HT of the mothers correlated with: newborn whole blood 5-HT (\( 0.743; p < 0.001 \)), maternal PLT (\( 0.590; p = 0.010 \)) and maternal \( \log \) PLT 5-HT (\( 0.794; p < 0.001 \)).

Linear regression models for PLT 5-HT and whole blood 5-HT of both newborns and their mothers were constructed by backward stepwise linear regression analysis. Indices that were dependent to either whole blood 5-HT (i.e. PLT 5-HT and PLT) or PLT 5-HT (i.e. whole blood 5-HT and PLT) were excluded. Gestational age (GA) and birth weight were included as additional variables. Newborn \( \log \) PLT 5-HT proved best explained (adjusted \( R^2 = 0.799 \)) by (beta, \( p \)-value) newborn Tryp (\( r = -2.010; p = 0.002 \)), maternal \( \log \) PLT 5-HT (\( r = 1.035; p = 0.016 \)) and newborn \( \log \) MPV (\( r = 1.750; p = 0.018 \)), when testing for newborn GA, newborn birth weight, newborn \( \log \) MPV, newborn whole blood Tryp and maternal \( \log \) PLT 5-HT. Maternal \( \log \) PLT 5-HT proved best explained (adjusted \( R^2 = 0.668 \)) by a constant \( [1.497 \text{ (std. error 0.452; } p = 0.005) ] \) and newborn whole blood 5-HT (\( 0.671; p < 0.001 \)) and newborn birth weight (\( -0.404; p = 0.012 \)) as indices, when testing for newborn GA, newborn birth weight, newborn whole blood 5-HT and newborn \( \log \) PLT 5-HT.

### 3.2. Relation between newborn PLT 5-HT and feeding mode

Of 20 included neonates, longitudinal PLT 5-HT and feeding data were available for only 5, since the majority of uncomplicated patients were most swiftly discharged from the NICU. Table 2 shows the characteristics of the examined patients at the time of admission. All were preterm. One (no 3) was small for gestational age ([20;23]; birth weight <10\(^{th}\) percentile for GA) and all had a low birth weight (<2.500 kg).
Table 2. Characteristics of the 5 longitudinally studied preterm neonates at the time of admission.

<table>
<thead>
<tr>
<th>no</th>
<th>gender</th>
<th>birth weight (g)</th>
<th>GA (weeks+days)</th>
<th>corrected GA* (weeks)</th>
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<td>01</td>
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<td>1740</td>
<td>32+5</td>
<td>33</td>
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<td>05</td>
<td>M</td>
<td>1780</td>
<td>30+6</td>
<td>30</td>
</tr>
</tbody>
</table>

M/F, male/female; GA, gestational age. Corrected GA is based on the Farr-Mitchell score which uses external characteristics at birth to estimate GA [23].

In this group (Figure) we observed 7 increases and 1 decrease of PLT 5-HT following institution of enteral feeding (i.e. in nos 1-5), and 2 decreases and 1 increase following institution of parenteral feeding, i.e. discontinuation of enteral feeding (respectively in nos 1, 4 and 5).

![Figure](image_url)

Figure. Courses of platelet 5-HT content in time in response to changes in feeding mode in 5 preterm born newborns.

For patient characteristics see Table 2. PLT 5-HT is expressed in nmol/10⁹ PLT. Feeding mode in indicated periods is shown by filled lines (parenteral), dashed lines (enteral) and dotted lines (enteral plus parenteral). All indicated changes in PLT 5-HT exceeded the reference change value (i.e. 21.6%), which implies that they reflect significance at the p<0.05 level. The changes of PLT 5-HT were in line with an increase upon enteral or enteral + parenteral feeding, and a decrease upon parenteral feeding (i.e. discontinuation of enteral feeding), except for 2 observations in patient no 5 (indicated by *).
The 2 observations that were inconsistent with our hypothesis occurred in a single patient (no 5), while in another (no 4) initial institution of enteral feeding and subsequent switches to parenteral and enteral feeding correlated perfectly with the expected course of PLT 5-HT. The courses of whole blood 5-HT in relation to changes in feeding mode (not shown) were similar to those of PLT 5-HT, but showed an even more pronounced pattern consistent with our hypothesis.

4. Discussion

We were interested to see whether PLT 5-HT is lower at the physiological condition of relative gut motor quiescence (i.e. newborns) and whether changes in feeding mode of newborns coincide with changes of PLT 5-HT. For this we compared PLT 5-HT of healthy term newborns (n=18) with that of their mothers (n=17) and linked early postnatal switches from enteral to parenteral feeding and vice versa to changes of PLT 5-HT in 5 preterm born infants. The most important observations were: newborns have about two times lower PLT 5-HT compared with their mothers, postnatal increases of PLT 5-HT coincide with the institution of enteral feeding, and postnatal decreases of PLT 5-HT coincide with the discontinuation of enteral feeding. The most important determinants of newborn PLT 5-HT seem to be newborn whole blood Tryp (suggesting a newborn PLT 5-HT depressing effect), newborn MPV and maternal PLT 5-HT (suggesting a newborn PLT 5-HT augmenting effect). A weakness of the present study is the inevitably low number of longitudinally investigated newborns, the inability to correlate PLT 5HT with a direct measure of GI motility and the use of RCV derived from apparently healthy adults.

We have no explanation for the inverse relation between newborn PLT 5-HT and newborn whole blood Tryp. In contrast to newborns, maternal whole blood Tryp proved not to be an important determinant of maternal PLT 5-HT. Maternal plasma Tryp decreases during pregnancy [24], while higher Tryp in umbilical venous plasma [25], compared with the mothers have previously been noted. The relation between newborn PLT 5-HT and newborn MPV seems conceivable, since large PLT may obviously harbor higher 5-HT contents at similar intracellular 5-HT concentrations, perhaps because of higher numbers of membrane-bound 5-HT transporters and intracellular 5-HT containing dense granules. Also remarkable were detectable levels of whole blood 5-HTP in 4/17 mothers and 11/18 newborns. This intermediate product in 5-HT synthesis is generally not detected in the blood of healthy adults by HPLC or in patients with carcinoid tumors [21], which adds to the notion of altered regulation of 5-HT synthesis in the fetus and/or the pregnant mother. 5-HTP has been detected before in pregnant women and their newborns [26] and several functions of 5-HTP in pregnancy have been suggested.
The about two times lower newborn PLT 5-HT than their mothers and the relation between newborn and maternal PLT 5-HT have been noted before [19;27-29]. Lower newborn PLT 5-HT has been ascribed to higher prenatal 5-HT catabolic activity, such as possibly originating from placental 5-HT degradation [27;29]. This option seems however less plausible, since 5-HT uptake by newborn PLT is considered to be fully functional [19], while at least some patients in the present study indicate that PLT 5-HT may remain below the maternal reference range up to at least 27 days postpartum (Figure). Differences between the intensities of newborn and adult GI motility seem more probable. The relation between newborn and maternal PLT 5-HT remains puzzling. Some have suggested heritability of PLT 5-HT as a cause [19]. Other possibilities are mutual influence of GI motility while any transplacental transport of 5-HT remains implausible because of the highly efficient mono- and diamine placental barrier [26;30].

PLT 5-HT half life amounts to 4.2 days and thereby equals approximately PLT half life, at least in adults [21]. This implies that each of the employed blood sampling intervals in our postnatal PLT 5-HT monitoring protocol (Figure) are well above the PLT 5-HT half life. The protocol therefore not only allowed detection of increases of PLT 5-HT deriving from increasing 5-HT exposure, but also detection of losses of PLT 5-HT content due to diminishing exposure of newly secreted PLT to 5-HT. Given the present time intervals we found that postnatal increases and decreases of PLT 5-HT were related to institution and discontinuation of enteral feeding, respectively, although the number of investigated neonates and observations was limited. Replication of our findings in larger groups is necessary, and preferably this study should be embedded in another longitudinal study in which blood is collected from healthy newborns. Our data nevertheless suggest that the mature postnatal PLT 5-HT content will at least partially become established by neurotransmission processes involved in GI motility. One of the investigated preterm infants (no 5, Figure) exhibited a pattern inconsistent with this notion. This patient was born at 30 gestational weeks, which is about two weeks prior to the time at which migrating motor complexes in the GI begin to appear [15]. It seems therefore possible that his inconsistent pattern of PLT 5-HT is related to an immature GI tract, and that such a condition may last up to 20 days after birth in preterm born infants.

In conclusion, consistent with relative gut motor quiescence we found that newborn PLT 5-HT is considerably lower as compared with their mothers. Early postnatal PLT 5-HT changes correlate with changes in feeding mode (which affect gut motor activity), such that institution and discontinuation of enteral feeding are linked to a PLT 5-HT increase and decrease, respectively. Further studies are
warranted to establish the link between PLT 5-HT and gut motor activity and the usefulness of PLT 5-HT in the detection and monitoring of GI pathology.

Acknowledgements
We thank Enge Venekamp and Dineke Fremouw for whole blood 5-HT analyses. The patients and their parents are greatly acknowledged for their participation in this study. The work was financially supported by the University Medical Center Groningen and the University of Groningen, Groningen, The Netherlands.
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