Perinatal growth restriction is not related to higher intestinal distribution and increased serum levels of 5-hydroxytryptamin in piglets

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ABSTRACT: Serotonin [5-hydroxytryptamin (5-HT)] is abundantly present in intestinal enteroendocrine cells and neurons and plays a crucial role in gastrointestinal functions (i.e., motility and mucosal secretion). Increased concentrations of 5-HT and its precursor L-Trp are present in plasma and brain tissues in case of intrauterine growth retardation (IUGR). Therefore, 5-HT might be involved in the impaired gastrointestinal function associated with IUGR. Small-for-gestational-age (SGA) piglets have been widely used as animal model for IUGR. Hence, the density of intestinal 5-HT cells in fetal and neonatal SGA piglets was compared with serotonergic cell density in normal weight (NW) littermates. Furthermore, 5-HT serum concentrations of the neonatal piglets were analyzed. Stereological analysis showed that fetal piglets have higher ($P<0.01$) volume densities of 5-HT enteroendocrine cells compared to 3-d-old piglets irrespective of BW. Serum concentrations did not differ in relation to postnatal age ($P=0.637$) and BW ($P=0.892$). These results contrast with serum and brain 5-HT and L-Trp levels in human and guinea pig SGA individuals and seemingly contest the fact that 5-HT plays an important role in gut impairment in SGA.

Key words: gastrointestinal system, perinatal, pig, serotonin, serum, small-for-gestational-age

INTRODUCTION

Mortality and morbidity of newborn piglets is an economic burden and threat to animal welfare. Perinatal mortality is especially high among small-for-gestational-age (SGA) piglets (Randall, 1972). Fetal growth restriction during late gestation, which results in SGA, can be attributed to placental insufficiency and impaired fetal gut functioning (Blakelock et al., 1998; Baserga et al., 2004). Thorough understanding of the mode of gut failure and to what extent it persists after birth is essential to evaluate curative and preventive strategies.

Serotonin [5-hydroxytryptamin (5-HT)] plays a major role in motility, secretion, and sensation of the gastrointestinal tract (Hansen, 2003). As such, increased plasma 5-HT in SGA infants can be linked to an impaired gut function (Manjarrez et al., 1998; Hernandez-Rodriguez et al., 2009). In mammals, the enteroendocrine cells of the gastrointestinal system are the major source of 5-HT (Erspamer, 1954). These cells already appear in the stomach wall in mid-gestational pig fetuses and show age-dependent regional differences (Van Ginneken et al., 2001). The latter finding suggests that maturation of the 5-HT system within the gastrointestinal tract occurs during the perinatal period. In addition, 5-HT could play different roles before and after birth. The presence of 5-HT in small intestinal tissue in relation to age and BW has not been investigated so far. As a result, the physiological relevance of 5-HT in perinatal development and gastrointestinal tract function in the pig remains incomplete. Therefore, the aim of this study was to assess the presence of 5-HT in small intestinal enteroendocrine cells and to determine 5-HT serum levels in perinatal piglets.

MATERIAL AND METHODS

Animals

Cross-bred fetal [70 to 80 (n = 10) and 90 to 105
Figure 1. Volume density (Vv) of 5-hydroxytryptamin (5-HT) immunoreactive cells according to age (PF = pig fetuses) in the proximal small intestine (Si prox) in small-for-gestational-age (SGA; white boxes) and normal weight (NW; black boxes) piglets (mean and 68% confidence interval).

d (n = 10) of gestation] and postnatal pigs [0 (n = 10) and 3 d (n = 10) of age; farm] were assigned to a normal weight (NW) group (mean litter BW ± 0.5 SD) or to a SGA group [BW ≤ (mean litter BW – 1.5 SD)]. All age groups contained 5 pairs of gender-matched pigs. Blood was collected from postnatal pigs by severing the carotid arteries after lethal barbiturate anesthesia. After an incubation period of 20 min at room temperature, the blood samples were centrifuged at 4°C at 1,500 × g. After dissection of all piglets, proximal small intestine biopsies were taken randomly; tissue was fixated for 2 h in 4% paraformaldehyde and routinely processed for paraffin embedding. The ethical committee on animal experimentation from the University of Antwerp approved the study.

Immunohistochemistry

After incubation with H2O2 (3%) and normal swine serum (20%; Dako), paraffin sections (4 μm) were incubated overnight (4°C) with a polyclonal rabbit anti-5-HT antibody (1/1000; Chemicon, Millipore, Billerica, MA). Sections were rinsed and incubated subsequently with a biotinylated swine anti-rabbit antibody and streptavidin-conjugated horseradish peroxidase (1/600, 2 h at room temperature; both from Dako). Immunoreactive cells were detected using 3,3′-diaminobenzidine (Dako).

Microscopic Evaluation

The volume density (Vv) of 5-HT immunoreactive (IR) cells was estimated using a point grid at 200x magnification. The Vv was calculated by the following equation: \[ V_v = \frac{\sum_{IR \text{ cells}}}{\sum_{epithelium}} \times \frac{P_{IR \text{ cells}}}{P_{epithelium}} \]

ELISA

Serum levels were measured by a 5-HT ELISA according to the manufacturer’s protocol (Enzo Life Sciences, Lorrach, Germany). All samples were analyzed in triplicate. Data are expressed as mean concentration ± SEM.

Table 1. The 5-hydroxytryptamin (5-HT) serum levels (mean and SEM) in postnatal (day 0 and 3) normal weight (NW) piglets and their small-for-gestational-age (SGA) littermates

<table>
<thead>
<tr>
<th>Age</th>
<th>NW 5-HT, ng/mL</th>
<th>SEM</th>
<th>SGA 5-HT, ng/mL</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>906.81</td>
<td>21.85</td>
<td>726.28</td>
<td>124.54</td>
<td>0.285</td>
</tr>
<tr>
<td>Day 3</td>
<td>862.00</td>
<td>127.33</td>
<td>996.68</td>
<td>237.6</td>
<td>0.732</td>
</tr>
</tbody>
</table>

Results and Discussion

The 5-HT immunoreactive cells were present in the small intestine both prenatally and postnatally. The enteroendocrine cells were scattered in the epithelia covering crypts and villi. The Vv of 5-HT enteroendocrine cells were not related to BW (P = 0.985). However, fetal pigs had higher (P < 0.01) volume densities compared to 3-d-old pigs in both NW and SGA piglets (Figure 1). This higher Vv in fetal small intestinal mucosa could merely be a morphological phenomenon caused by an increase in mucosal tissue volume per surface area after birth (Van Ginneken et al., 2002). Nevertheless, these higher fetal densities of enteroendocrine cells may contribute to a higher bioavailability of 5-HT, where it could act both as a growth factor and neurotransmitter on the developing enteric nervous system, in which, at least at the level of the stomach, 5-HT immunoreactivity was not seen in the fetal stages (Van Ginneken et al., 2001).

Similar to the density of 5-HT enteroendocrine cells, the serum levels of 5-HT did not reveal age-related (P = 0.637) or BW-related (P = 0.892) differences in the postnatal pigs (Table 1). This contrasts with the higher l-Trp levels in plasma of human intrauterine growth retardation infants (Manjarrez et al., 1998; Hernandez-Rodriguez et al., 2009), albeit that 5-HT plasma levels were not related with the levels of its precursor (Manjarrez et al., 1998).

In conclusion, both 5-HT ELISA and stereology analysis did not show BW-related differences up to 3 d.
However, in the present study 5-HT IR was also observed in the enteric plexuses (data not shown). Therefore, alterations in neuronal 5-HT content may contribute to the impaired intestinal motility seen in SGA infants (Robel-Tillig et al., 2002). To strengthen these findings, the neuronal presence of 5-HT needs to be further elucidated.

LITERATURE CITED


