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Sex and intrauterine growth restriction modify brain neurotransmitters profile of newborn piglets

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ABSTRACT

The current study aimed to determine, using a swine model of intrauterine growth restriction (IUGR), whether short- and long-term neurological deficiencies and interactive dysfunctions of Low Birth-Weight (LBW) offspring might be related to altered pattern of neurotransmitters. Hence, we compared the quantities of different neurotransmitters (catecholamines and indoleamines), which were determined by HPLC, at brain structures related to the limbic system (hippocampus and amygdala) in 14 LBW and 10 Normal Body-Weight (NBW) newborn piglets. The results showed, firstly, significant effects of sex on the NBW newborns, with females having higher dopamine (DA) concentrations than males. The IUGR processes affected DA metabolism, with LBW piglets having lower concentrations of noradrenaline at the hippocampus and higher concentrations of the DA metabolites, homovanillic acid (HVA), at both the hippocampus and the amygdala than NBW neonates. The effects of IUGR were modulated by sex; there were no significant differences between LBW and NBW females, but LBW males had higher HVA concentration at the amygdala and higher concentration of 5-hydroxyindoleacetic acid, the serotonin metabolite, at the hippocampus than NBW males. In conclusion, the present study shows that IUGR is mainly related to changes, modulated by sex, in the concentrations of catecholamine neurotransmitters, which are related to adaptation to physical activity and to essential cognitive functions such as learning, memory, reward-motivated behavior and stress.

1. Introduction

Intrauterine growth restriction (IUGR), or fetal growth restriction, is defined as the failure of the fetus to reach its potential growth-rate and is mainly due to insufficient supply of nutrients and oxygen, which causes Low Birth-Weight (LBW) offspring. Traditionally, IUGR has been related to maternal malnutrition (which induces nutrient shortage to the fetus; Bell and Ehrhardt, 2002; Wu et al., 2004) and/or maternal hypobaric hypoxia in case of pregnant women living or visiting high-altitudes (which induces oxygen shortage to the fetus; Keyes et al., 2003). IUGR incidence at low-altitude is estimated in around 6 and 15% for affluent and less-favored areas, respectively (Baschat, 2004; Moore et al., 1998). The combination of high-altitude and scarcity is related to IUGR rates around 17% (Jensen and Moore, 1997; Mortola et al., 2000). However, there is also a concerning increase in IUGR occurrence under adequate social, environmental and nutritional conditions in developed countries. More than 60% of IUGR offspring in developed countries are triggered by placental insufficiency due to abnormal placental development (Ghidini, 1996; Krebs et al., 1996), which impairs nutrient and oxygen transfer to the fetus and, hence, adequate growth.

The growth deficiencies related to shortage of oxygen and nutrients are typically asymmetrical, since fetuses are able to adapt their physiology to optimize or preserve the growth of vital organs, like the brain (effect known as 'brain-sparing'), at the expense of others (Yu and Upadhyay, 2004). The objective of the 'brain-sparing' effect is to protect the development of the brain (Miller et al., 2016), the most relevant organ for survival, to ensure essential functions necessary for life; the so-called 'autonomic functions' like breathing, recognition the surrounding environment and suckling (Perry,

Abbreviations: DA, dopamine; HVA, homovanillic acid; IQ, intelligence quotient; IUGR, intrauterine growth restriction; LBW, low birth-weight; NBW, normal body-weight.

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2002). However, this does not guarantee normal brain development and function, thus there is still an increased risk of short- and long-term neurological deficiencies, specifically at the limbic system of the IUGR offspring, which cause cognitive, emotional and interactive impairments (Geva et al., 2012; Miller et al., 2016). These alterations in case of IUGR may occur due to changes in the neurotransmitters of limbic system (Bauer et al., 2003), catecholamines and indoleamines are among the most important (Berumen et al., 2012; Palmiter, 2007).

Hence, there is a strong necessity for research in the area for the future development of diagnostic, preventive and therapeutic strategies to diminish the incidence and consequences of IUGR. However, there is a paucity of studies, except a recent experiment in rabbits (Hernández-Andrade et al., 2015), evaluating the profiles of different excitatory and inhibitory neurotransmitters at the limbic system, which play a major role in autonomic functions.

Swine is a recognized model for IUGR caused by deficiencies in placental growth (Gonzalez-Bulnes et al., 2016); its high prolificacy limits the available uterine space for the development of the placenta and, hence, compromises placental functions and causes IUGR in a high percentage of the littermates (around 15–20%; Ashworth et al., 2001; Foxcroft et al., 2006; Wu et al., 2006). The experimental advantage is therefore the coexistence of IUGR and normal fetuses in the same litter, which allows accurate comparative studies among them, like in other polytocous species; but, specifically IUGR piglets represent a suitable model for IUGR humans due to developmental similarities in central autonomic functions (Gootman, 1986). Moreover, pigs have a more similar brain development to humans than rodent and rabbits (Bassols et al., 2014; Lind et al., 2007). Hence, the aim of this study was to evaluate possible differences between Normal and Low Birth-Weight (NBW and LBW, respectively) newborn piglets in the levels of different neurotransmitters (catecholamines and indoleamines) at hippocampus and amygdala; two structures of the limbic system, related to learning, memory, reward-motivated behavior and stress.

2. Material and methods

2.1. Animals and experimental procedure

The trial was performed under a Project License from the INIA Scientific Ethic Committee and involved 24 piglets of Iberian x Duroc genotype selected by sex and birth-weight from the components of the litters of 21 multiparous (fourth pregnancy) Iberian sows. Distribution of Normal and Low Birth-Weight newborns was performed on the basis of birth-weight under a standard deviation (SD; Anthony et al., 2003; Blomberg et al., 2010) to the mean birth-weight of the piglets from all litters (1319 ± 313 g). Hence, 10 piglets were NBW (5 females and 5 males) whilst 14 were LBW (8 females and 6 males). Piglets were sacrificed immediately after birth by stunning and exsanguination according to the Spanish Policy for Animal Protection RD53/2013, which meets the European Union Directive 2010/63/UE about the protection of animals used in experimentation. Afterwards, piglets were decapitated and, after weighing the head, the brain was immediately removed from the skull and also weighed. Hippocampus (n = 24, 10 NBW and 14 LBW) and amygdala (n = 22, 10 NBW and 12 LBW) were dissected, snap frozen in liquid nitrogen and finally stored at −80 °C until neurotransmitter quantification.

2.2. Analysis of neurotransmitters

Samples were weighted and homogenized by sonication (Branson Digital Sonifier 250, Branson Ultrasonics Corp., Danbury, CT) in a lysis buffer (150 mM NaCl, 50 mM Tris-HCl and 0.1% Triton X-100) with 0.3 mg tissue/1 mL lysis buffer relation. Internal standards of catecholamines and indoleamines (Dihydroxybenzylamine (DHBA) and Nωmetil-5-hydroxytryptamine (Nω) respectively) were added on lysis buffer to allow the comparison between runs. After that, HPLC buffer (0.25 M perchloric acid containing 0.1 M sodium metabisulfite ($Na_2S_2O_5$) and 0.25 M ethylenediaminetetraacetate (EDTA)) were added in a 1.5 dilution. Afterwards, samples were centrifuged at 12000*g* for 10 min at 4 °C and 40 μL of supernatant was injected in HPLC for determination of catecholamines (dopamine [DA] and its metabolites noradrenaline [NA], 3,4-dihydroxyphenylacetic acid [DOPAC] and homovanillic acid [HVA]) and indoleamines (serotonin [5-HT] and its metabolite 5-hydroxyindoleacetic acid [5-HIAA]).

Concentrations of the different neurotransmitters were determined using high performance liquid chromatography (Elite LaChrom, Merck-Hitachi, Prague, The Czech Republic) equipped with a Cromolith Rp-18e column (Merck, Darmstadt, Germany) with electrochemical detection (ESA Coulochem II 5200). The mobile phase consisted of 0.5 M citrate buffer pH 2.8, 0.05 mM EDTA, 1.2 mM sodium octyl sulphate (SOS) and 1% acetonitrile. The applied voltage was set at 0.4 mV and the flow rate was 1 mL/min.

2.3. Statistical analysis

Effects of sex (female *vs*. male) and occurrence of IUGR (LBW *vs*. NBW) and their interactions on developmental traits (total body-weight, total weights of head and brain and relative weights of head-to-body and of brain-to-head and brain-to-body) and neurotransmitters concentrations were assessed by a *t*-Student test after a Kolmogorov-Smirnov test showed normality of the data. Results were expressed as mean ± SEM and the threshold for statistical significance was set at $P < 0.05$.

3. Results

3.1. Morphometric features of piglets

Mean body-weight was higher in NBW than in LBW piglets (Table 1), but there were no significant differences in body-weight between female and male piglets, neither in the NBW group nor in

Table 1
Absolute and Relative Weights in neonate piglets.

	Treatment				SEM	P-value
	NBW-F	NBW-M	LBW-F	LBW-M		IUGR
Body-weight (g)	1318	1316	643	546	83.2	<0.0001
Head-weight (g)	261.0	256.0	155.0	142.0	12.8	<0.0001
Head- to Body- weight ratio	0.20	0.20	0.25	0.26	0.007	<0.0001
Brain-weight (g)	31.4	31.0	27.6	27.8	0.55	0.001
Brain- to Body-weight ratio	0.03	0.03	0.05	0.05	0.003	<0.0001
Brain- to Head-weight ratio	0.12	0.13	0.18	0.20	0.008	<0.0001

F = Female, M = Male, NBW = Normal birth weight, LBW = Low birth weight.