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Impact of colostrum and plasma immunoglobulin intake on hippocampus structure during early postnatal development in pigs

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ABSTRACT

The first milk, colostrum, is an important source of nutrients and an exclusive source of immunoglobulins (Ig), essential for the growth and protection from infection of newborn pigs. Colostrum intake has also been shown to affect the vitality and behaviour of neonatal pigs. The objective of this study was to evaluate the effects of feeding colostrum and plasma immunoglobulin on brain development in neonatal pigs.

Positive correlations were found between growth, levels of total protein and IgG in blood plasma and hippocampus development in sow-reared piglets during the first 3 postnatal days. In piglets fed an elemental diet (ED) for 24 h, a reduced body weight, a lower plasma protein level and a decreased level of astrocyte specific protein in the hippocampus was observed, as compared to those that were sow-reared. The latter was coincident with a reduced microgliogenesis and an essentially diminished number of neurons in the CA1 area of the hippocampus after 72 h. Supplementation of the ED with purified plasma Ig, improved the gliogenesis and supported the trophic and immune status of the hippocampus.

The data obtained indicate that the development of the hippocampus structure is improved by colostrum or an Ig-supplemented elemental diet in order to stimulate brain protein synthesis and its development during the early postnatal period.

1. Introduction

It is well documented that intake of the first milk, colostrum, in addition to providing essential nutrients, also provides passive immunity (immunoglobulins) to prevent infections as well as growth factors, thereby playing an essential role in the growth and survival of newly born ungulates (Tlaskalova-Hogenova et al., 1994; Alonso-Spilsbury et al., 2005). Hence, during the first few days of life, all necessary immunoglobulins and immune factors must be transferred over the 'open' gut mucosa to the blood circulation of the neonate (Markowska-Daniel et al., 2010; Foisnet et al., 2010). The highest concentration of immunoglobulins is in the first colostrum. Pigs withheld from colostrum ingestion during the first few hours of their life, often die due to the development of diarrhoea – which is supposedly caused by pathogens. Generally, it is accepted that the cause of death is bacterial diarrhoea and general infection due to impaired passive immunity transfer from the mother to the neonate (Gomez et al., 1998). In ungulates this transfer possibly only occurs through the gut mucosa, from ingested colostrum to the neonate. Colostrum is rich in hormones and other biologically active peptides "designed" to be transferred directly to the blood via the "open" gut during the first 24 h of life (Milon et al., 1983; Foisnet et al., 2010). Thus, the colostrum not only provides the neonate with nutrients, it also ensures proper gut development (the process of gut closure after 24 h of birth) (Jensen et al., 2001; Siggers et al., 2011). Past research has shown that full development of the gut functions (e.g., gut motility, absorption and

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secretion) occurs mainly in the early postnatal period and that the colostrum is an important factor in this process (Xu, 1996; Mubiru and Xu, 1997; Marion et al., 2002; Woliński et al., 2003; Korczynski et al., 2006).

It is a common observation that piglets receiving more colostrum are stronger, more alert and have an enhanced suckling behaviour, compared to their weaker, colostrum-deprived, counterparts (Weström et al., 1987; Koldovský, 1995; Zabielski et al., 1998; Rauprich et al., 2000). Moreover, the behavioural parameters directly indicate that the brain status and function can be affected by the colostrum. Protection of the brain from inflammatory insult is the top priority in the first few weeks of life (Choi et al., 2010). So, there is an increasing interest in the potential effects of the colostral compounds on brain development. Only a few studies have evaluated such effects to reveal that the colostrum-borne macromolecules absorbed into the systemic circulation, e.g., lactoferrin, transferrin, IgG, and epidermal growth factor-like proteins are also transported into the CSF (cerebrospinal fluid), in a time-dependent manner through the blood-CSF or blood-brain barrier (Mubiru and Xu, 1997; Harada et al., 2002). CSF flows through the ventricular system, passing over all the regions of germinal activity and contains growth factors and other neurotropic factors, which are important for neuron survival and proliferation, as well as brain development. Even though, the above mentioned information all points towards the colostral components being able to directly affect the fast developing, growing brain, we did not find any data describing the type of association between colostrum rearing and the development of cognitive function (behaviour) in newborn pigs.

In the present study, we chose to study the hippocampus, the brain structure thought to be responsible for memory and learning, more specifically the CA1 area. We focused on the possible immunochemical and morphological relations between colostrum deprivation and the development of hippocampal structures. The main aim of our study was to evaluate the role of colostrum and its most pronounced component–Ig, on the development of the hippocampus in newborn pigs.

2. Materials and methods

2.1. Animals

The care and use of animals was conducted in accordance with the principles outlined in the current Guide to the Care and Use of Experimental Animals and was approved by the Local Ethics Review Committee on Animal Experiments in the Malmö/Lund region, Sweden.

The experiment was conducted on cross-bred ((Yorkshire × Swedish Landrace) × Hampshire) pigs (*Sus scrofa domesticus*) obtained from the closed herd belonging to the Swedish University of Agricultural Sciences (Odarslöv research farm, Department of Agricultural Biosystems and Technology, Alnarp, Sweden), where complete management, production and health records are maintained. A total of 63 piglets from 6 litters, born on time and with no complications, were used in the study. Immediately after birth and before the first suckling, the piglets were removed from the sow and placed in a clean, straw-bedded area, under a heating lamp. The piglets were then weighed and divided into 5 experimental groups with randomly chosen pairs of males and females from different litters: newborn, un-suckled controls (NB, $n = 7$); suckled pigs which stayed with their sow for 24 or 72 h (Sow, $n = 14$,); or experimentally fed piglets given either milked colostrum (Col, $n = 14$), an elemental diet (ED, $n = 14$), or the elemental diet supplemented with purified plasma immunoglobulins (ED + Ig, $n = 14$). The pigs were gavage-fed these experimental diets, 10 ml/kg body weight, via a stomach tube every 2 h for up to 24 h (12 feedings). After this, half of the piglets, 7 from each feeding group, were euthanized (at 24 h) while the other half of the piglets, independently of group, were further fed with the ED every 2 h, for up to 72 h. During the feeding experiments, the piglets were housed together in a clean stable area, with an ambient temperature of between 28 and 29 °C, with a dry towel for bedding.

Piglets from the NB group were euthanized just after birth. At 24 or 72 h after the start of the experiment, piglets from the fed groups were euthanized, i.e., 4 from each group for fresh brain sampling (left and right hippocampus, $n = 8$) and 3 pigs for the brain morphology after in situ fixation (left and right hippocampus, $n = 6$).

2.2. Diets

Aliquots of about 1000 ml of the first milk, colostrum (Col), were hand-milked from 5 sows during farrowing, pooled and stored fresh at 4 °C during the feeding experiment. Samples of the colostrum pool were centrifuged at 20,000 × *g* for 60 min at 4 °C, the lipid layer at the top and the bottom pellet were removed, and the remaining colostrum supernatant was then stored at −20 °C until biochemical analysis.

The elemental diet (ED), contained glucose, free amino acids (Kabiven®), lipids in the form of emulsified soybean oil (Vitalipid adult) and vitamins (Soluvit®, all Fresenius Kabi AB, Uppsala, Sweden), as used in hospitals for total parental nutrition. To make the ED iso-energetic with the sow milk, the pigs received 120 ml ED/kg/day, which corresponds to an energy intake of 0.451 MJ/kg/day.

The ED was supplemented with purified porcine plasma immunoglobulins, 33.5 mg/ml (ED + Ig) in one experimental group. Plasma Ig was purified by ammonium sulphate precipitation (Grodzki and Berenstein, 2010). A pool of citrated blood (500 ml), obtained from about 10 slaughtered pigs at a local slaughterhouse (Scan AB, Kristianstad, Sweden) was centrifuged at 3000 × *g* for 15 minutes, using a refrigerated centrifuge. The blood plasma was separated and solid $(NH_4)_2SO_4$ was added to a final concentration of 38%. After an overnight incubation, the precipitate was obtained by centrifugation, washed in 38% $(NH_4)_2SO_4$ solution, dissolved in distilled water and finally dialyzed against distilled water. The plasma Ig preparation was stored frozen until used.

2.3. Colostrum and blood analyses

At euthanasia of the piglets, blood from the left or right subclavicular vein was collected (2 ml) into commercially available EDTA-treated tubes (Becton & Dickinson) with the addition of 500 KIE/ml of the protease inhibitor aprotinin (Trasylol™, Bayer Health Care, Germany). After centrifugation at 3000 × *g* for 10 minutes, using a refrigerated centrifuge, plasma was harvested and frozen at −20 °C for later analysis.

Total protein concentration (mg/ml) in the plasma samples and colostral supernatant was determined according to Lowry et al. (1951), using BSA (fraction V, Sigma Chemicals) as the standard.

The level of immunoglobulin G (mg/ml) in plasma samples and colostral supernatant was analyzed by single radial immunodiffusion (Fahey and McKelvey, 1965), using specific antibodies to porcine IgG produced in rabbits (Carlsson et al., 1980) and purified porcine IgG as the standard (Sigma).

2.4. Brain sampling for morphology

The pigs were anesthetized using 0.5–1.5% Fluothane (Zeneca, Gothenburg, Sweden) in a mixture of air and O_2, at approximately 0.5–1 L/min. The brain was in situ fixated by transcardial perfusion with 4% formaldehyde in 0.1 M phosphate buffer, pH 7.4. The brain was then isolated and the hippocampus dissected and post fixed in the same fixative solution overnight at +4 °C. The next day the hippocampus was cut into 50-μm-thick frontal slices using a vibratome Vibroslice 752 M (Campden Instruments Ltd, Great Britain). Hippocampal slices were rinsed out with 0.1 M phosphate buffer, pH 7.4 and treated with blocking solution (1% normal goat serum and 0.3% Triton X-100). Double and triple immunofluorescence staining of hippocampal slices was performed. Neurons were identified by monoclonal antibodies, specific to neuronal nuclear protein (NeuN). Polyclonal antibodies against glial fibrillary acid protein (GFAP – specific astrocyte marker) were used for astrocyte detection. Iba1 (ionized calcium binding adaptor molecule 1) was used as the marker for microglial cells. Slices were incubated with primary mouse anti-NeuN antibodies (Merck Millipore, USA), diluted in PBS, pH 7.4 (1:1000), chicken anti-GFAP antibodies (diluted 1:1500) (Abcam, USA) and rabbit anti-Iba1 polyclonal antibodies (diluted 1:1500) (WAKO, Japan) for 16 h at +4 °C. After rinsing, slices were then incubated with secondary antibodies; anti-mouse conjugated with Alexa Fluor 488 (1:1000), anti-chicken conjugated with Alexa Fluor 647 (1:1000) and anti-rabbit conjugated with Alexa Fluor 555 (1:1000) (Invitrogen, USA) for 1.5 h at room temperature. The slices were then rinsed, placed on histological slides and mounted with Fluorescence Mounting Media (Dako, Denmark). Images of hippocampal tissue were analyzed with a confocal FV1000-BX61WI microscope (Olympus, Japan). Estimation of the amount of neurons and microglial cells on images obtained was carried out using UTHSCSA Image Tool software (version 3, University of Texas, San Antonio, TX, USA), by counting the number of NeuN- and Iba-1-positive cells respectively, in equal squares of the different areas within the CA1 hippocampal area, which were limited by the counting frame (650 × 650 px test area). The number of cells per unit area was then calculated.

2.5. Brain sampling for biochemical analyses

After anaesthesia by an overdose of mebumal (Sigma, USA), the pigs' brains were quickly dissected out and the hippocampi were isolated and immediately frozen. The tissue was then homogenized in 10-volumes of 25 mM Tris–HCl buffer, pH7.4, containing 1 mM EDTA, 2 mM dithiothreitol, 0.2 mM PMSF and 0.01% merthiolate, at +4 °C. The homogenates were centrifuged at 100,000 × *g* for 60 minutes at +4 °C. The supernatant containing the water-soluble protein fraction was used to analyze the cytosolic forms of the astrocyte specific proteins, glial fibrillary acid protein (GFAP)