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Research Report

Effects of early weaning and social isolation on the expression of glucocorticoid and mineralocorticoid receptor and 11β-hydroxysteroid dehydrogenase 1 and 2 mRNAs in the frontal cortex and hippocampus of piglets

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ABSTRACT

Pigs weaned at young ages show more abnormal and aggressive behaviors and cognitive deficits compared to later weaned pigs. We investigated the effects of age, weaning and/or social isolation on the expression of genes regulating glucocorticoid response [glucocorticoid receptor (GR), mineralocorticoid receptor (MR), 11β-hydroxysteroid dehydrogenases 1 and 2 (11β-HSD1 and 11β-HSD2)] in the frontal cortex and hippocampus. Early- (EW; $n = 6$) and conventionally-weaned (CW; $n = 6$) piglets were weaned at 10 and 21 days after birth, respectively. Non-weaned (NW) piglets of both ages (NW; $n = 6$/group) remained with their dams. Immediately before euthanasia, half of CW, EW and NW animals were socially isolated for 15 min at 12 (EW, NW) and 23 (CW, NW) days of age. Differences in amounts of 11β-HSD1, 11β-HSD2, GR and MR mRNA were determined by quantitative real-time RT-PCR and data subjected to multivariate linear mixed model analysis. When compared with NW piglets at 12 days of age, the hippocampi of EW piglets showed decreased gene expression ($P < 0.01$). Social isolation decreased gene expression ($P < 0.05$) in the frontal cortex of all piglets. Twelve-day-old piglets showed higher MR mRNA in the frontal cortex ($P < 0.01$) and lower 11β-HSD2 and GR mRNA ($P < 0.05$) in the hippocampus compared to 23-day-old animals. Results indicate that EW affected the hippocampus of piglets at 12 days of age, while social isolation affected frontal cortex regardless of age. These results may be correlated with behavioral and cognitive changes reported in EW piglets.

1. Introduction

Early maternal separation has been extensively studied and shown to cause long-term psycho-physiological effects on brain and behavior of humans and animals (Kuhn and Schanberg, 1998; Sanchez et al., 2001). Maternal separation interferes with the proper development of both psychobiological and neuroendocrine regulatory mechanisms in the developing brain (Kanitz et al., 2004; Kaufman et al., 2000; Plotsky et al., 2001). Moreover, maternal separation affects the

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individual's behavior by eliminating the predictability and controllability provided by the mother–infant interaction (Plotsky et al., 2001). The early weaning of piglets can provide a useful model for studying early maternal separation effects on behavior and neuroendocrine mechanisms. Previous studies revealed that piglets weaned at young ages, for instance, at less than 21 days of age show abnormal behaviors, such as belly-nosing (Gardner et al., 2001; Orgeur et al., 2001) and intensified aggression (Hohenshell et al., 2000; Yuan et al., 2004) later in life when compared to piglets weaned at later ages. Early-weaned piglets, when exposed to 15 min of social isolation, also show cognitive deficits compared to non-socially isolated animals (Laughlin and Zanella, 2002; Souza and Zanella, 2004). The behavioral outcomes and cognitive deficits reported may result from modifications in responsiveness of neuronal mechanisms during a sensitive period of brain development (Plotsky et al., 2001; Tuchscherer et al., 2004). An age-dependent sensitivity to environmental manipulations (Hemsworth and Barnett, 1992; Hemsworth et al., 1986) and brain exposure to stress hormones, such as corticosteroids, has been reported to occur in piglets during the first weeks after birth (Weaver et al., 2000).

Hippocampus and frontal cortex functions include cognition and behavioral organization (Cao et al., 2004; Kesner et al., 1996, 2004; Ongur and Price, 2000). Basal concentrations of endogenous corticosteroid hormones like glucocorticoids (GC) are essential for maintaining these functions (Erickson et al., 2003). However, hippocampus and frontal cortex can be affected by stressful events since both areas contain high density binding sites for corticosteroids. Thus, high concentrations of corticosteroids in the hippocampus during brain development cause behavioral and cognitive deficits that may not be reversible (McEwen, 1997), as well as cognitive deficits in the frontal cortex by altering neuronal morphology and function (Cook and Wellman, 2004; Mizoguchi et al., 2004).

Stressful challenges, such as early weaning and social isolation, are responsible for activation of the stress axis resulting in increased GC levels (Hay et al., 2001; Hohenshell et al., 2000; Kanitz et al., 2004). One of the primary outcomes of increased central GC action is to trigger a negative feedback system which suppresses further activation of the hypothalamic–pituitary–adrenal (HPA) axis, preventing serious neuronal damage directly caused by GC (Lee et al., 2002). Therefore, at early brain developmental stages, the disruption of the GC negative feedback system, which is characterized by decreased GR expression in the hippocampus and frontal cortex, may be responsible for brain dysfunction (Mizoguchi et al., 2003, 2004). The GC action is mediated by mineralocorticoid (MR) and glucocorticoid receptors (GR) which bind GCs with different affinities in the brain (Erickson et al., 2003). Mineralocorticoid receptors, when activated, buffer the activation of the stress response by preventing GC from binding to GR (Perreau et al., 1999), which once heavily bound mediates various neuronal changes (Herman, 1993; McEwen, 1997). Glucocorticoids indirectly regulate the activity of the enzymes 11β-hydroxysteroid dehydrogenases type 1 (11β-HSD1) and type 2 (11β-HSD2), which modulate GC action (Holmes et al., 2003). When acting as dehydrogenases, both enzymes protect the brain from the deleterious effects of excessive GC by rendering them inactive (Seckl, 1997). Thus, during early stages of development, the disruption of the HPA axis may compromise the developing brain and be accountable for some aspects of cognitive impairment and abnormal behaviors (Mizoguchi et al., 2003, 2004).

Therefore, our research focus is to understand the molecular events associated with the cognitive deficits reported previously in early-weaned piglets subjected to social isolation, a short-term stressor (Laughlin and Zanella, 2002; Souza and Zanella, 2004). We hypothesize that spatial and social cognitive impairments experienced by early-weaned piglets are preceded by aberrant expression of stress-sensitive genes in the frontal cortex and hippocampus. Our aim was to investigate the effects of weaning at two ages, 10 and 21 days, on 11β-HSD1, 11β-HSD2, GR and MR mRNA abundance in the frontal cortex and hippocampus of piglets, when the animals were either socially isolated or kept with their littermates.

2. Results

The piglets studied in this experiment were weaned at two ages. Early-weaned animals were weaned at 10 days of age and samples collected at 12 days of age. Conventionally-weaned animals were weaned at 21 days after birth, and frontal cortex and hippocampus collected at 23 days of age. When examining mRNA levels for 11β-HSD1, 11β-HSD2, GR and MR using real-time RT-PCR, a significant effect of weaning on gene expression was observed only in the hippocampus of early-weaned animals (interaction of age by weaning; $P = 0.004$). Early-weaned piglets showed suppressed expression of the four tested genes in the hippocampus by an average fold of –2.52 ($P = 0.004$), when compared with non-weaned piglets at 12 days of age (Table 1). Conversely, no significant changes in expression of the four genes were detected in the hippocampus of conventionally-weaned piglets when compared with non-weaned piglets at 23 days of age (Table 1). Interestingly, when examining the mRNA abundance for 11β-HSD1, 11β-HSD2, GR and MR in the frontal cortex, there were no evidences of significant differences in mRNA levels in either early-weaned

Table 1 – Average change in relative expression of stress-responsive genes in the frontal cortex and hippocampus of weaned compared with non-weaned piglets

Brain area	Weaning status	Average fold change	SEM (limits)	P value
Frontal cortex	EW	1.35	[0.913, 1.914]	NS
	CW	–1.48	[–2.196, –0.808]	NS
Hippocampus	EW	–2.52	[–3.337, –1.244]	<0.01
	CW	1.50	[1.1299, 1.904]	NS

Note. Positive and negative values indicate higher and lower gene expression, respectively, in weaned compared with non-weaned piglets at 12 and 23 days of age (EW = early-weaned, CW = conventionally weaned). SEM = standard error of the mean. NS = not significant.