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Effects of maternal deprivation on the adrenocorticotrophic and gonadotrophic axes in the hypothalamo–pituitary unit of preweanling female sheep: The histomorphometric approach

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Abstract

This study was designed to investigate the histochemical effects of maternal deprivation on the adrenocorticotrophic and gonadotrophic axes in the hypothalamo–pituitary unit of preweanling lambs. Twelve-week-old female lambs were divided into either the control (lambs reared under undisturbed maternal conditions; $n = 3$) or the maternally deprived group (lambs separated for three days from their dams; $n = 3$). The corticotrophin-releasing hormone (CRH) and gonadotrophin-releasing hormone (GnRH) in the median eminence and the adenohypophyseal adrenocorticotrophin (ACTH), gonadotrophins (LH and FSH) and mRNAs for their β-subunits were investigated using the immunohistochemistry or hybridohistochemistry. In maternally deprived lambs, the percentage of the area occupied by immunoreactive (ir)-CRH nerve terminals was lower ($P < 0.05$) and the percentage of the adenohypophyseal area (PAA) occupied by ir-ACTH cells was higher ($P < 0.05$) compared with the control lambs. In the hypothalamo–gonadotrophic axis of maternally deprived lambs the percentage of area occupied by ir-GnRH nerve terminals was higher ($P < 0.05$) and the PAA occupied by ir-FSHβ cells was lower ($P < 0.05$) in comparison with controls. The PAA occupied by gonadotrophs detected using hybridohistochemistry was higher ($P < 0.05$) for LHβ-mRNA in contrast to a lower ($P < 0.05$) percentage for FSHβ-mRNA in maternally deprived lambs compared with those staying with dams. In conclusion, maternal deprivation affected the accumulation of CRH and ACTH. The different and more striking alterations in FSH synthesis and storage in comparison with those concerning LH were observed in maternally deprived lambs. Thus, rupture of the preweanling young-mother social contact can affect the gonadotroph population activity, especially that relating to FSH-producing cells in the infantile female sheep.

Keywords: Maternal deprivation; CRH; Adrenocorticotrophic cells; GnRH; Gonadotrophic cells; Histochemistry; Lamb

1. Introduction

In mammals, maternal deprivation or social isolation produces psychobiological disturbances. The neuroendocrine, endocrine and behavioural consequences of disrupting mother–infant contact have been investigated mainly in rodent pups and young primates. The recent study of Kanitz et al. (2004) provided evidence that early disruption of mother–piglet interactions may cause effects on the activity of the hypothalamic-pituitary-adrenal (HPA) axis, and on the immune-brain circuitry of young. In maternally deprived lambs, very scanty data relate to behavioural alterations, hormonal and immune consequences and reduced growth (Orgeur et al., 1999; Napolitano et al., 2003).

Weaning is associated with a rupture of the mammalian mother–infant social bond. Under natural conditions, lambs grazing with ewes on the pasture undergo the weaning process gradually and can consume a solid diet as well as suckle their dams. The natural rearing period of sheep breeding in grasslands lasts from birth to weaning at about 4 months of age. Total weaning may results in the stress of maternal

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deprivation, i.e. psychoemotional and in consequence physiological disturbances of homeostasis. These effects may be treated as the natural mechanisms of transition from the infantile to the juvenile period of postnatal ontogeny.

The activation of the hypothalamo–adrenocorticotrophic axis, involving the corticotrophin-releasing hormone (CRH) and adrenocorticotrophin (ACTH), is the main defining feature of neural and endocrine adaptations known as the stress response (Marti and Armario, 1997). Thus, the pituitary adrenocorticotrophic cells, as the site of synthesis, storage and release of ACTH, make the morphological base of the endocrine effector mechanism of the stress response. The mammalian HPA system is under maternal regulation. The frustration arising from maternal feeding deprivation resulting in elevated plasma cortisol levels was observed in lambs prevented from suckling their mothers during an early postnatal period (Napolitano et al., 2003). In rodents, even at the time of weaning (Schmidt et al., 2002) or beyond the time of weaning (Hennessy et al., 1995), when pups are capable of self-feeding and no longer require tactile stimulation to induce eliminative functions, maternal deprivation results in a disregulation of the HPA axis at multiple levels (Marti and Armario, 1997; Hennessy et al., 1995). However, the precise neuroendocrine mechanisms implicated must yet be determined.

It is generally considered that the stress of both physical and emotional origin affects the mammalian reproductive system via actions at many levels of the hypothalamic–pituitary–gonadal (HPG) axis. Nonetheless, the predominant impact is on the activity of the hypothalamo–pituitary unit relating to gonadotrophin-releasing hormone (GnRH) and gonadotrophins—luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Thus, the pituitary gonadotroph population, as the site of synthesis, storage and release of LH and/or FSH, is the morphological basis of the effector mechanism of neuroendocrine functions of the reproductive system. Stressors generally suppress the secretory activity of the hypothalamo–gonadotrophic axis and reduce gonadal functions in ewes during anoestrum (Przekop et al., 1988), a premating period (Domey et al., 1973), and estrus (Przekop et al., 1984). Concerning HPG axis activity, there is no evidence for any changes in maternally deprived mammals. Thus, there is a need to know if and how maternal deprivation affects the intrapituitary mechanisms underlying the secretion of gonadotrophic hormones during the transition from the infantile to the juvenile period of postnatal ontogeny.

The present work was designed to describe effects of the maternal deprivation on the: (i) CRH and GnRH in nerve terminals of median eminence, and (ii) ACTH, LH, FSH or mRNAs for LHβ and FSHβ in the adenohypophyseal corticotroph or gonadotroph populations of the preweanling female lamb. The effects of maternal deprivation on the storage and/or synthesis of hormones were evidenced by their immunoreactive or in situ hybridised contents. Comparison of the adenohypophyseal populations of cells sythesizing or storing LH and FSH, between maternally deprived and control lambs, may help to describe the nature of the effector mechanism of neuroendocrine infantile/juvenile switch during the postnatal sexual development.

2. Materials and methods

2.1. Animals and tissue preparation

The study was performed on Polish Merino female lambs born in the first week of July on the Bieganowo Farm (Poland); the experiment was conducted on the same farm. Lambs were left to graze with ewes on pasture for twelve weeks after parturition. Twelve-week-old lambs (average weight 18.3 ± 1.17 kg) were divided before weaning randomly into either the control ($n = 3$; average weight 18.5 ± 0.71 kg) or maternally deprived ($n = 3$; average weight 18.5 ± 1.50 kg) group. These lambs were preweaning but independent of their mothers for feeding. Control lambs grazed with dams on the pasture and suckled their mothers one to two times per 24 h. Maternally deprived lambs were strictly separated from dams. They were reared in partial isolation from the herd in the group of three on the fenced part of pasture (approximately 600 m^2) for a period of 72 h. They had a direct contact among themselves and an inspected audiovisual and olfactory contact with their herd (staying beyond the fence, on the same pasture) but not with their dams. Thus, this was the partial social contact utterly deprived of maternal tactile and psychoemotional stimulation. Maternally deprived lambs grazed pasture—were weaned onto solid food alone and were allowed free access to water. After that, animals were subjected to 8 min loading and 5 min transportation (approximately 1 km in a livestock trailer with internal floor dimensions of 3.5 m × 2 m). Instantly after transportation to a local licensed slaughterhouse and after unloading lambs were anesthesized with an i.v. injection of pentobarbitone sodium (20 mg/kg; Biochemie GmbH, Kundl, Austria). Thus, lambs were slaughtered by decapitation at 73 h/74 h after the beginning of the experiment. All procedures were approved by the Local Ethics Committee in Warsaw, according to the Polish Law for the Care and Use of Animals (2 August 1997).

Immediately after decapitation, brains were perfused via both carotid arteries with 1000 ml 0.1 M phosphate buffered saline (PBS; Sigma, St. Louis, USA) and subsequently with 1500 ml 0.1 M PBS containing 4% (w/v) paraformaldehyde (Sigma–Aldrich, Seelze, Germany) and 15% saturated picric acid (MERCK, Darmstadt, Germany) solution (w/v), pH 7.4. The hypothalami and pituitaries were dissected 20 min after the beginning of perfusion and postfixed for 72 h by immersion in the same fixative and washed with 0.01 M PBS. All solutions used for the following steps of tissue preparation and fixation were made up fresh and sterile autoclaved. Hypothalami jointly with the median eminence and pituitary stalk were cryoprotected in a 20% sucrose solution in 0.1 M PBS for at least two days at 4 °C. Hypothalami were frozen at −10 °C and sectioned in frontal planes into 10 μm sections on a cryostat (Jung CM 1500, Leica Instruments GmbH, Nussloch, Germany). Pituitaries were dehydrated in graded alcohol, embedded in paraplast (Sigma, St. Louis, USA) and then 4 μm sections were cut in the sagittal plane.

2.2. Immunohistochemistry

The immunoreaction for: CRH, GnRH, ACTH, LHβ, FSHβ was developed by immunohistochemical (IHC) technique. Brain sections were washed in 0.1 M PBS, incubated for 4 h in 1% hydrogen peroxide in 0.1 M PBS and 24 h in 3% preimmune normal lamb serum in 0.1 M PBS. Pituitary sections were deparaffined, rehydrated, washed in 0.01 M PBS, incubated for 30 min in 2% preimmune normal lamb serum in 0.01 M PBS and 30 min in 0.1% hydrogen peroxide (Chempur, Piekary Śląskie, Poland) in 0.01 M PBS. Brain sections were incubated with primary antisera raised in the rabbit: anti-sheep (2–10) GnRH No. 1900 diluted 1:1000 and anti-sheep CRH No. R567 diluted 1:1500 for 8 days at 4 °C. Pituitary sections were incubated with primary antisera raised in the rabbit: anti-sheep LHβ No. I3 diluted 1:1000, anti-sheep FSHβ No. P5 diluted 1:200 and anti-sheep $ACTH_{17–39}$ diluted 1:800 for 4 days at 4 °C. All antibodies, except anti-sheep CRH, were prepared in INRA (Nouzilly, France) and CNRS (Gif sur Yvette, France). Methodological details of their preparation and their specificity were described by Dubois and Barry (1974), Hurbain-Kosmath et al. (1990) and Dubois (1971). The anti-sheep CRH