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Combined effects of perfluorooctane sulfonate (PFOS) and maternal restraint stress on hypothalamus adrenal axis (HPA) function in the offspring of mice

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ABSTRACT

Although it is known that prenatal exposure to perfluorooctane sulfonate (PFOS) can cause developmental adverse effects in mammals, the disruptive effects of this compound on hormonal systems are still controversial. Information concerning the effects of PFOS on hypothalamus adrenal (HPA) axis response to stress and corticosterone levels is not currently available. On the other hand, it is well established that stress can enhance the developmental toxicity of some chemicals. In the present study, we assessed the combined effects of maternal restraint stress and PFOS on HPA axis function in the offspring of mice. Twenty plug-positive female mice were divided in two groups. Animals were given by gavage 0 and 6 mg PFOS/kg/day on gestation days 12–18. One half of the animals in each group were also subjected to restraint stress (30 min/session, 3 sessions/day) during the same period. Five plug-positive females were also included as non-manipulated controls. At 3 months of age, activity in an open-field and the stress response were evaluated in male and female mice by exposing them to 30 min of restraint stress. Male and female offspring were subsequently sacrificed and blood samples were collected to measure changes in corticosterone levels at four different moments related to stress exposure conditions: before stress exposure, immediately after 30 min of stress exposure, and recuperation levels at 60 and 90 min after stress exposure. Results indicate corticosterone levels were lower in mice prenatally exposed to restraint. In general terms, PFOS exposure decreased corticosterone levels, although this effect was only significant in females. The recuperation pattern of corticosterone was mainly affected by prenatal stress. Interactive effects between PFOS and maternal stress were sex dependent. The current results suggest that prenatal PFOS exposure induced long-lasting effects in mice.

Introduction

The perfluoroalkyl acids (PFAA) and their salts, such as perfluoroalkyl sulfonates, perfluoroalkyl carboxylates, and telomer alcohols, are chemicals that have wide consumer and industrial applications, including protective coatings for fabrics and carpets, paper coatings, insecticides, paints, cosmetics, and fire-fighting foams (Paul et al., 2009). The industrial production of perfluorooctane sulfonate (PFOS) and its derivatives stopped in 2000, and the European Union banned most uses from the summer of 2008. However, hundreds of related chemicals: homologues with shorter or longer alkyl chain, perfluorooctanoic acid (PFOA) and telomers, which potentially may degrade to perfluoroalkanoic (carboxylic) acids, are not regulated (Jensen and Leffers, 2008). In recent years, a number of studies involving perfluorinated compounds (PFCs) have been focused on increasing the general knowledge on the toxicity, environmental distribution and fate, as well as the potential human health risks of exposure to these pollutants, especially PFOS and PFOA (Calafat et al., 2006, 2007; Negri et al., 2008; Kato et al., 2009; Toms et al., 2009). However, significant gaps still exist on that knowledge. Thus, information from the scientific literature concerning health risks of dietary exposure to these compounds, potential adverse effects of their presence in drinking water and safety limits, or data on PFC levels in human tissues other than blood and the public health implications this can mean, is very limited. Information on the behavioral, reproductive and developmental effects of PFCs in mammals, or their possible interactions with other pollutants or adverse situations, such as stress, has been, until recently, rather scarce.

In 2006, we initiated in our laboratory a wide program focused on increasing the available information and overall understanding of the toxicity and health risks of PFCs, paying especial attention for PFOS, the most extensively distributed and studied. On the one hand, we investigated if the diet, including drinking water, could mean a significant contribution to human exposure to PFCs, as well as the role that food processing and packaging could play as a source of PFCs through dietary intake (Ericson et al., 2008a,b, 2009a,b). We also

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determined the levels of PFCs in human blood, milk and liver of subjects belonging to the same population for which dietary exposure to these pollutants was determined (Kärrman et al., 2009). Although a correlation between dietary intake and blood levels of PFOS was noted, our current results did not justify food intake as the main route of exposure governing blood concentrations of other PFCs (Ericson et al. 2008a, 2009b). On the other hand, we investigated if the maternal and developmental effects of PFOS could be modified by concurrent administration of restraint stress, whereas the behavioral effects of PFOS in adult mice were also assessed (Fuentes et al., 2006, 2007a,2007b,2007c).

Recently, Fei et al. (2009) have suggested that in humans, PFOA and PFOS exposure at plasma levels seen in the general population might reduce fecundity. Such exposure levels are common in developed countries. However, in a previous study of the same researchers, no convincing associations between developmental milestones in early childhood and levels of PFOA or PFOS, as measured in maternal plasma early in pregnancy, were found (Fei et al., 2008). This result was in agreement with the conclusion of a study by Washino et al. (2009) showing that in utero exposure to relatively low levels of PFOS was negatively correlated with birth weight. On the other hand, although possible PFOS effects (Austin et al., 2003) on hypothalamus adrenal axis (HPA) have been also suggested, according to the scientific literature this has been poorly studied. Moreover, the results of recent studies have implicated developmental PFOS exposure with alterations in sexual hormone levels (Ishibashi et al., 2007; Liu et al., 2007) and on developmental immune function in rodent and avian models, suggesting that PFOS modulates certain immunological functions in offspring following exposure to this chemical during gestation (Keil et al., 2008; Peden-Adams et al., 2007, 2008, 2009). The HPA axis could be implicated in this modulation.

There is increasing evidence indicating that environmental factors, which are present early in life, can result in an increased vulnerability to disease in adults. Several physiological systems are programmed early in life, affecting the subject response to possible adverse conditions in adults. In this sense, one of the most studied systems has been the HPA axis and the environmental early programming of this system (Weinstock, 2008). We recently found some evidence indicating that PFOS could alter corticosterone levels in mice (Fuentes et al., 2006). To better understand the implication of PFOS on the HPA axis system, to investigate the response of the HPA axis after stress exposure, as well as its recuperation pattern could be of interest. Therefore, the aim of the present study was to assess the combined effects of maternal restraint stress and PFOS on HPA axis function in the offspring of mice.

Materials and methods

Animals. Adult male and female Charles–River CD1 mice (28–32 g) were obtained from Criffa (Barcelona, Spain). After a quarantine period of 7 days, females were mated with males (2:1) until copulation was detected. The day on which a vaginal plug was found was designated as day 0 of gestation. Animals were housed in plastic cages in a climate-controlled facility at a temperature of 22 ± 2 °C, a relative humidity of $50 \pm 10\%$, and a constant day–night cycle (light: 08:00–20:00 h) with free access to food (Panlab rodent chow, Barcelona) and tap water. From gestation day (GD) 0 to GD 18, two or three dams were housed together. On GD 18, dams were individually housed to allow individual litter observation during delivery and weaning until postnatal day (PND) 21. Pups were then separated according to the respective treatment group and sex. They were maintained in the same housing conditions until the beginning of the behavioral tests. At 3 months of age, general activity and anxiety like behavior of male and female mice were evaluated in an open-field. One week after behavioral testing, mice were stressed and sacrificed at different time points to collect blood in order to determine corticosterone levels. The use of animals and the experimental protocol were approved by the Animal Care and Use Committee of the "Rovira i Virgili" University (Tarragona, Spain).

Chemicals. Perfluorooctane sulfonate (PFOS, potassium salt) was purchased from Fluka Chemical (Steinheim, Switzerland). It was dissolved in 0.5% Tween 20 (Bio-Rad, Hercules, CA, USA) and administered by gavage at doses of 0 and 6 mg/kg/day from GD 12 to 18. Control mice received the vehicle only. PFOS solutions were weekly prepared and concentrations were adjusted to be administered at volumes of 0.30 ml/30 g body weight. The selection of the PFOS doses was based on the results of previous studies performed in our laboratory (Fuentes et al., 2006, 2007a).

Treatment. On GD 0, female mice were weighed and randomly assigned to one of the 5 experimental groups ($n = 5$ animals per group). Animals were given by gavage 0 and 6 mg PFOS/kg/day on GD 12–18. One-half of the animals in each group were subjected to restraint stress during the same period. Five plug-positive females were also included as non-manipulated controls. Restrained animals were immobilized three times per day (30 min each time). Mice were subjected to the first restraint session immediately after PFOS administration. Subsequently, a fixed time gap of 3 h was established between sessions. The restraint procedure consisted of placing the mice in metacrilate cylindrical holders from Letica Scientific Instruments (Panlab, Barcelona) and maintaining them in a prone position for 30 min. According to the results of previous studies, this procedure produces stress in pregnant rodents (Darnaudéry et al., 2004; Fuentes et al., 2007a,2007b). A maximum of two randomly selected males and two females of each litter were evaluated ($n = 10$ animals per group).

Behavioral testing. On PND 3, maternal care was evaluated. At 3 months of age, general activity and anxiety like behavior were evaluated in an open-field test.

Maternal care. On PND 3, pups and dam were separated, and the time required by the dam to collect all pups, the number of lickings, the number of times in lactating behavior, and the quality of the nest were recorded.

Open-field activity. General motor activity was measured in an open-field apparatus, consisting of a wood 47×47 cm square surrounded by a 40 cm-high light colored wall. A 10-cm area near the surrounding wall was delimited and considered as the periphery, while the rest of the open-field more than 10 cm far from the wall, was considered as the center area. During the test, mice were allowed to move freely around the open-field during 30 min. The path and movements of the animals were recorded by a video camera (Sony CCD-IRIS model), which was placed above the square. The video tracking program Etho-Vision® (Noldus Information Technologies, Wageningen, The Netherlands) was used to measure the total distance traveled and the number of rearings (as a measure of vertical activity).

Time-course of the corticosterone release after a mild stress condition. At 3 months of age, the stress response was evaluated in male and female mice by exposure to 30 min of restraint stress. Male and female pups were exposed to restraint during 30 min and then sacrificed by decapitation in order to collect blood samples to measure corticosterone levels at 4 different time points. A set of 10 males and 10 females per group of treatment were used for each of the 4 time points selected. A fixed schedule for the experiment was designed. All animals at the basal condition were sacrificed between 10:00 and 10:30 AM, mice under the stress condition were sacrificed after 30 min of restraint (between 10:30 and 11:00 AM), while animals in the two recuperation conditions (early and late recuperation) were sacrificed 60 and 90 min after the beginning of restraint stress exposure (between 11:00 and 11:30 AM, and between 11:30 and 12:00 AM, respectively).