Developmental exposure to polychlorinated biphenyls PCB153 or PCB126 impairs learning ability in young but not in adult rats

Boix Jordi, Piedrafita Blanca, Cauli Omar and Felipo Vicente

Laboratory of Neurobiology, Centro de Investigacion Príncipe Felipe, Avda Autopista del Saler, 16, 46013 Valencia, Spain

Introduction

Polychlorinated biphenyls (PCBs) are a family of 209 industrial chemicals which are persistent organic pollutants and are toxic and endocrine disrupters. They accumulate in the food chain and humans are exposed to PCBs through diet. PCBs are present in human blood and milk [1]. Children born from mothers exposed to PCBs show memory deficits and cognitive dysfunctions as well as sensory and motor disorders [2], indicating that developmental exposure to PCBs is neurotoxic and affects cerebral function. PCBs have been classified according to their structure in coplanar and non-coplanar types, depending on the position of the benzene rings, and also into 'dioxin-like' (among the coplanar) and 'non-dioxin-like' based on their toxicological profile. For instance, the dioxin-like PCBs, such as PCB126, bind to the Ah-receptor, producing endocrine disruption in experimental animals, as with dioxins, and are considered to be far more toxic than non-dioxin-like PCBs. However, non-dioxin like PCBs (such as PCB153) have been described as carcinogens or interfering in neurotransmitter release [3]. It is unclear whether the cognitive effects of developmental exposure to PCBs are similarly expressed at different ages or are preferentially expressed during youth or maturity. The molecular mechanisms by which developmental exposure to PCBs affect cerebral and cognitive functions remain unclear. NMDA receptors play a crucial role in some types of learning. Activation of the glutamate– NO–cGMP pathway modulates some forms of learning [4]. The aim of the present study was to assess whether exposure of rats to a dioxin-like PCB (PCB126) or to a non-dioxin-like PCB (PCB153) during pregnancy and lactation affects the ability of the pups to learn the Y maze task when the rats are young (3 months) or adult (8 months). We have also assessed whether impairment of learning ability is associated with altered function of the glutamate–NO–cGMP pathway in brain in vivo and whether the effects are similar or different in males and females.

Material and Methods

Developmental exposure of rats to PCB153 or PCB126 Pregnant female Wistar rats (Charles River Laboratories) were treated orally with PCB153, PCB126 or vehicle (controls) from gestational day 7 to postnatal day 21. PCBs were dissolved in corn oil and administered daily mixed in a sweet jelly bit (Transgel_ from Charles River Laboratories). The treatments were as follows: PCB153 (2,2¢,4,4¢,5,5¢-pentachlorobiphenyl, from Fluka, 99.8% purity) 1 mg/kg body weight per day; PCB126 (3,3¢,4,4¢,5- pentachlorobiphenyl, from Larodan, 99.9% purity), 100 ng/kg body weight per day. Rats in the control group were treated daily with vehicle (Corn oil). The dams were housed individually. Pups were weaned at 21 days of age and housed two or three animals per cage until the experiments were performed. Learning tests and microdialysis studies were performed when these pups were 3 or 8 months old. Both male and female rats were used and the results were analysed separately to assess whether there is some gender-associated difference. All animal procedures were approved by our Institute and met the guidelines of the European Union for care and management of experimental animals (86/609/EEC).

Results and Discussion

We first tested whether developmental exposure to PCB153 or PCB126 affects the ability of rats to learn the Y maze task in young (3 months old) rats. As shown in Fig. 1A, the number of trials required to learn the task was significantly higher ($P < 0.001$) in 3-month-old male rats exposed to PCB153 or PCB126 than in control rats. Similar results were obtained with female rats (Fig. 1B).. We also tested learning ability in 8 month-old rats exposed or not

to PCB153 or PCB126. These data are also shown in Fig. 1. At 8 months of age the number of trials required to learn the task was similar in control rats and in those exposed developmentally to PCB153 or PCB126. Similar results were obtained with female rats (Fig. 1B). In control rats the number of trials required to learn the task was significantly ($P < 0.001$) higher at 8 months of age than at 3 months of age. However, in rats exposed to PCB153 or PCB126 the number of trials required to learn the task was similar at 3 or 8 months of age (Fig. 1) We then tested whether the function of the glutamate–NO–cGMP pathway in brain in vivo is also affected by exposure to PCB153 or PCB126 in young rats. Administration of NMDA through the microdialysis probe activates the glutamate–NO–cGMP pathway and increases the formation of cGMP and its release to the extracellular fluid. In 3-month-old male rats addition of NMDA significantly increased extracellular cGMP in all groups of rats. However, the function of the pathway (the increase in cGMP induced by NMDA) was significantly ($P < 0.001$) impaired in 3-month-old male rats exposed to PCB153 or PCB126 than in control rats.

We also tested the function of the glutamate–NO–cGMP pathway in brain in vivo when the rats were 8 months old. In control male rats, the function of the pathway was significantly impaired ($P < 0.001$) at 8 months than at 3 months of age. In 8-month-old male rats perinatally exposed to PCB153 or PCB126 the function of the pathway in cerebellum in vivo was not different from control rats.(Fig. 3A). Similar results were obtained for female rats (Fig. 3B). Moreover, these data also show that the function of the pathway decreases in 8-month-old control rats compared with 3- month-old control rats. However, the function of the pathway was not different at 3 months and 8 months of age for rats perinatally exposed to PCB153. For rats perinatally exposed to PCB126, the function of the pathway was also similar at 3 months or 8 months of age.

The results obtained show that:

1 Exposure to PCB153 or PCB126 during gestation and lactation impairs the ability to learn the Y maze task when the rats are young (3 months old) but not when they are adult (8 months old).

2 Developmental exposure to these PCBs also impairs the function of the glutamate–NO–cGMP pathway in cerebellum in vivo in young but not in adult rats.

3 For the parameters studied (learning of the Y maze task and function of the glutamate–NO–cGMP pathway in cerebellum in vivo), the effects of PCB153 and of PCB126 are similar in males and females.

4 Both the non-dioxin-like PCB153 and the dioxin-like PCB126 impair learning of the Y maze task and the function of the glutamate–NO–cGMP pathway in cerebellum in vivo; however, PCB126 induces these effects with a daily intake by the mother of 100 ng/kg while for PCB153 the intake was 1 mg/kg per day.

This indicates that the two PCBs can induce similar effects but that the dioxin-like PCB126 is 10 000-fold more potent than the non dioxin- like PCB153. In control rats the function of the glutamate–NO–cGMP pathway and learning ability are lower in adult than in young rats. These age-related differences are not present in rats exposed to PCBs[5].

We believe that impairment of learning is a direct consequence of impaired function of the glutamate–NO–cGMP pathway, which modulates learning of this Y maze task. Additional mechanisms by which perinatal exposure to PCBs and impairment of the function of the glutamate–NO–cGMP pathway may lead to altered learning ability can be also speculated. It has been postulated that cognitive impairment induced by PCBs reflects an altered pattern of neuronal connectivity due to alterations in the ontogenic profile of dendritogenesis. PCB-induced alterations in NO–cGMP metabolism may contribute to altered neuronal connectivity. NO acts as a modulator of neurite development, slowing neurite outgrowth and modulating the formation of axonal projections[6]. NO is also involved in refinement of synaptic connections. During synaptic remodeling, NO acts as a signal for synaptic detachment and inhibits synaptic formation by cGMP-dependent and independent mechanisms. Alterations by the PCBs of NO–cGMP metabolism may contribute to altered neuronal connectivity and learning ability by affecting any of the above steps. An additional factor that may mediate the impairment of learning by PCBs is oxidative stress. Exposure to PCBs induces the formation of reactive oxygen species and alters dopaminergic neurotransmission[7]. Oxidative stress may also alter glutamatergic neurotransmission and cognitive function.

The fact that perinatal exposure to PCBs impairs in parallel manner the function of the glutamate–NO–cGMP pathway and the ability to learn the Y maze task in young rats further supports the idea that the function of this pathway modulates this type of learning[8]. There is also a parallel decrease in the function of the pathway and in the

ability to learn this task in adult compared with young normal rats[9]. All these studies support the suggestion that the function of the glutamate– NO–cGMP pathway modulates this type of learning. The impairment of learning found here in young rats developmentally exposed to the PCBs would therefore be a consequence of the impaired function of the pathway. The lack of impairment of the pathway in adult rats exposed to the PCBs would explain the lack of effects on learning ability at 8 months. The functions of the pathway and learning ability in young (3 months old) rats exposed to the PCBs is similar to those of adult control rats (8 months old), and, in contrast to control rats, these functions do not decrease further between 3 and 7 months of age. This suggests that developmental exposure to the PCBs accelerates the effects of aging or maturation on the function of the pathway and, subsequently, on the ability to learn this task. These results suggest that perinatal exposure to PCBs impairs cognitive function when the rats are young but the that effect is reduced in adult rats[10].

The mechanisms by which maturation or exposure to the PCBs impairs the function of the pathway remain unclear. The fact that perinatal exposure to PCB126 or PCB153 impairs signal transduction associated with NMDA receptors and learning ability in a similar manner in young male and female rats supports the idea that these PCBs induce neurotoxic effects by mechanisms that are independent of their role as endocrine disrupters. Moreover, these neurochemical alterations seem to occur at low levels of exposure. This indicates that, in addition to the endocrine disrupting activity, the PCBs also have neurotoxic effects, which could occur at exposure levels possibly lower than those required to alter endocrine function.

The impairment of the function of the glutamate–NO–cGMP pathway induced in youth by developmental exposure to the PCBs could be one of the mechanisms contributing to the cognitive impairment found in children whose mothers ingested PCB contaminated food during pregnancy and lactation[11]. The identification in detail of the mechanisms by which the PCBs impair the function of the pathway could serve to help design possible treatments to reverse the impairment of the pathway and of learning ability.

Acknowledgements

This work was supported by grants from the European Commission program Food Quality & Safety, FOOD-CT-2003-506143, and from the Ministerio de Educación y Ciencia (SAF2002-00851, SAF2004-0001-E and SAF2005-06089) and from the Ministerio de Sanidad (Red G03-155 and FIS PI050253) of Spain and by grants from Conselleria de Empresa, Universidad y Ciencia (Grupos03 ⁄ 001, GV04B-055, GV04B-012, GVS05 ⁄ 082, ACOMP06⁄005) and AP005⁄06 from Conselleria de Sanitat of Generalitat Valenciana.

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Fig.1: The ability to learn the Y-maze conditional discrimination task is reduced in young rats perinatally exposed to PCB153 or PCB126. It also decreases with age in control rats but not in rats perinatally exposed to PCB153 or PCB126

A MALES	Number of trials to learn	
Control 3-months-old	**52 ± 3**	
PCB 153 3-months-old	**77 ± 2**	**p< 0,001**
PCB 126 3-months-old	**93 ± 7**	**p< 0,001**
Control 8-months-old	**97 ± 10**	
PCB 153 8-months-old	**87 ± 7**	**n.s.**
PCB 126 8-months-old	**114 ± 15**	**n.s.**

B FEMALES	Number of trials to learn	
Control 3-months-old	**58 ± 7**	
PCB 153 3-months-old	**110 ± 4**	**p< 0,001**
PCB 126 3-months-old	**94 ± 6**	**p< 0,001**
Control 8-months-old	**115 ± 18**	
PCB 153 8-months-old	**118 ± 20**	**n.s.**
PCB 126 8-months-old	**83 ± 15**	**n.s.**

Fig.2: Perinatal exposure to PCB153 or PCB126 impairs the function of the glutamate–NO–cGMP pathway in brain in vivo in rats at 3 months of age.

A MALES	NMDA-induce increase in extracellular cGMP (% of Basal)	
Control	**882 ± 120**	
PCB 153	**506 ± 102**	**p< 0,001**
PCB 126	**355 ± 51**	**p< 0,001**

B FEMALES	NMDA-induce increase in extracellular cGMP (% of Basal)	
Control	**864 ± 70**	
PCB 153	**388 ± 121**	**p< 0,001**
PCB 126	**197 ± 58**	**p< 0,001**

Fig. 3: Perinatal exposure to PCB153 or PCB126 does not impair the function of the glutamate NO–cGMP pathway in brain in vivo in rats at 8 months of age

A MALES	NMDA-induce increase in extracellular cGMP (% of Basal)	
Control	**397 ± 78**	
PCB 153	**229 ± 82**	**n.s.**
PCB 126	**292 ± 72**	**n.s.**

B FEMALES	NMDA-induce increase in extracellular cGMP (% of Basal)	
Control	**272 ± 57**	
PCB 153	**272 ± 64**	**n.s.**
PCB 126	**238 ± 42**	**n.s.**