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Subacute oral exposure to benzo[α]pyrene (B[α]P) increases aggressiveness and affects consummatory aspects of sexual behaviour in male mice

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ABSTRACT

Benzo[α]pyrene (B[α]P) is a neurotoxic pollutant which is also able to affect some behaviour and cognitive function. Here we report that a subacute oral exposure to B[α]P increases aggressiveness and affects copulatory behaviour in male mice. Indeed, after 3 weeks of exposure to B[α]P at 0.02 and 0.2 mg/kg, we have observed that B[α]P 0.02 mg/kg-treated male mice are more aggressive than control mice in resident-intruder test because a significant decrease in the latency time of the first attack and a significant increase in the number of attacks in B[α]P 0.02 mg/kg-treated mice were found. On the other hand, we have found that subacute exposure (4 weeks) to B[α]P, does not affect the appetitive aspects and sexual motivation in copulatory behaviour because the latency to the first mount between control and B[α]P-treated male mice was not significantly different. We have nevertheless, surprisingly found that B[α]P (0.02–0.2) mg/kg-treated mice have performed significantly more sexual behavioural acts including mounting, intromission latency and intromission frequency than control mice. Although these last results suggest that B[α]P improves the consummatory aspects of sexual behaviour, we cannot conclude that this neurotoxic pollutant has advantage of sexual function because B[α]P has been shown to alter the monoaminergic neurotransmitter system and causes endocrine dysregulation *via* toxic effect.

1. Introduction

Currently, benzo[α]pyrene (B[α]P) is the most widely studied polycyclic aromatic hydrocarbon (PAH), showing a highly toxic potential with various harmful effects including atherogenesis, teratogenesis and carcinogenesis [1–4]. The hematotoxicity, nephrotoxicity and neurotoxicity can also result from an exposure to B[α]P [3,5]. Exposure of humans to B[α]P is unavoidable especially by the oral route (e.g., consumption of contaminated food and water) and inhalation route (e.g., urban air) which are the principal ways of contamination by this xenobiotic [6,7]. It is widely accepted that this environmental xenobiotic induces several types of biochemical alterations in the body which may lead to various pathologies [8–11]. The capacity of B[α]P to cross the blood–brain barrier [12,13] and to provoke brain oxidative stress [14] raise the question of the possible adverse effects of exposure to B[α]P on behaviour. Indeed, oxidative stress can alter overall brain activity including neurotransmission and cause neuronal cell death [14–17]. Currently, there is increasing evidence that brain oxidative stress can provoke behavioural disturbances [14,17]. In this sense, several recent reports have examined a close relationship between oxidative stress and some behaviours such as anxiety levels [17–20], pathological anxiety [21,22] and depression [23,24]. The neurotoxic action of B[α]P on nervous system function has been first examined by Jayasekara et al. [9] in mice and afterwards by Stephanou et al. [8] in rats. These authors found dramatic neurochemical alterations in the brain monoaminergic system including catecholamine and serotonin levels in several brain areas of B[α]P-exposed rodents [8,9] suggesting that B[α]P may also lead to behavioural and hormonal disturbances [8]. Grova et al. [13,25] described that B[α]P induced biochemical changes in the murine brain by impairing the expression of *N*-methyl-D-aspartate (NMDA) receptors implicated in cognitive function, anxiety among others. Recently, we found that B[α]P impacts neuronal receptor gene expression including 5-hydroxytryptamine (serotonin) 1A ($5HT_{1A}$) and mu 1-opioid (MOR_1) in lactationally B[α]P-exposed pups. Additionally, we found that the neurobiological effects of B[α]P during lactation were associated with disturbances in the postnatal neurodevelopment of pups and both behaviour and cognitive function of young adult mice [26]. Despite these evidences, the putative effects of B[α]P on adult mammalian behaviour have not received much attention except few recent reports [5,13,14,25,27]. It has been reported that B[α]P at high doses (20–200 mg/kg) disturbs anxiety level occurring an anxiolytic-like profile while at low doses (0.02–0.2 mg/kg) this

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environmental chemical compound alters short-term learning and spatial memory capacities in the Y-maze and the Morris water maze tests in female mice [13,25,27]. These last findings are in agreement with the observations of Majchrzak et al. [28] who reported short-term memory disorders in workers of a coke processing plant in Poland, and those of Otto et al. [29] who mentioned learning disorders in children exposed during early life to high levels of PAHs in the Czech Republic. It is worth mentioning that these populations were likely also exposed to volatile organic compounds, such as benzene, toluene and xylene that are also potent neurotoxins [30–32].

Prompted by the previous data showing that B[α]P induces neurochemical changes in the brain [8,9] and related behavioural disturbances [5,13,14,25–27], we were interested to evaluate the effects of B[α]P on aggressiveness and sexual behaviour. For these objectives, the adverse effects of subacute oral exposure (3 weeks) to B[α]P on male mice aggressive behaviour have been examined in the resident-intruder test. Furthermore, the negative effects of subacute oral exposure (4 weeks) to B[α]P on male mice copulatory behaviour have been also investigated in sexual behavioural test.

2. Materials and methods

2.1. Animals

We used Swiss albino male mice (OF1), 9 weeks old at the time of reception from the breeder (Charles River, France) ranging in weight from 30 to 40 g. The animals were housed individually in transparent plastic cages (24 cm × 12 cm × 8 cm) with a 12-h light:12-h dark schedule (lights on at 8:00 p.m.) with free access to water and food (SDS Dietex, France) and maintained at a constant temperature (21 ± 2 °C) and a relative humidity of 55 ± 10%. Experiments began after a 2-week period of acclimatization. All animal procedures were carried out in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC).

2.2. Drugs and treatment

B[α]P and estradiol benzoate were purchased from Sigma–Aldrich Co. (St. Quentin Fallavier, France). The following drugs and dosages were used: B[α]P (0.02 and 0.2 mg/kg) dissolved in avocado oil (Cauvin, France) and avocado oil (control mice).

Nine mice were randomly assigned to each one of the experimental groups receiving 0.02, and 0.2 mg/kg of B[α]P, and avocado oil alone (control mice). Each animal received a daily oral administration of B[α]P over a 28-day period (subacute period). Dosages were given 60 min before testing. We have chosen to treat mice with B[α]P by the oral route rather than the intraperitoneal route because the former pathway and inhalation are the principal routes of contamination by this xenobiotic [6,7].

On day 21, the effects of B[α]P on the aggressive behaviour were evaluated by using the resident-intruder test.

On day 28, the effects of B[α]P on the copulatory behaviour were evaluated by using the male sexual behavioural test.

2.3. Behavioural study

2.3.1. Resident-intruder test

After 3 weeks of B[α]P treatment, the aggressive encounters were observed in the home cage of the tested male (resident) when the intruder male was exposed to the resident [33]. During the dark phase (2 h after lights off) of the light/dark cycle under red dim light, animal behaviours including the latency time to the first attack and the number of attacks performed by the resident were videotaped during 5 min with camera positioned above the testing cage.

2.3.2. Sexual behavioural test

Prior to treatment with B[α]P, naive male mice were familiarized with a first set of females to select those exhibiting normal sexual behaviours until displaying copulatory behaviour. After 4 weeks of B[α]P treatment, the male sexual behavioural test was performed [34,35]. Male copulatory behaviour was measured during a 30-min behavioural test with a Swiss female mouse in the male's home cage during the dark phase (2 h after lights off) of the light/dark cycle under red dim light. The experimental female mice were brought into sexual receptivity by administration of estradiol benzoate (5 μg/0.05 ml oil, subcutaneous, once daily) for 4 days prior to the tests [34,36].

For each male, the latency to the first mount, intromission latency, mounting and intromission frequency were recorded during a 30-min with camera positioned above the testing cage.

2.4. Statistical analysis

Behavioural data were analyzed by ANOVA followed by Fisher test. Data are reported as mean ± SEM. Level of significance was set at $p < 0.05$. All statistical analyses were carried out using the Statview® 4.5 statistical package (Abacus Concepts, Inc.).

3. Results

3.1. Subacute effect of B[α]P on male mice aggressive encounters in resident-intruder test

3 weeks of treatment of male mice with B[α]P induced a significant decrease in the latency time of the first attack ($p < 0.01$) and a significant increase in the number of attacks ($p < 0.05$) in B[α]P 0.02 mg/kg-treated mice compared to control mice (Fig. 1). No significant differences were found between B[α]P 0.2 mg/kg-treated mice and control mice with respect to the aggressive behaviour ($p > 0.05$) (Fig. 1).

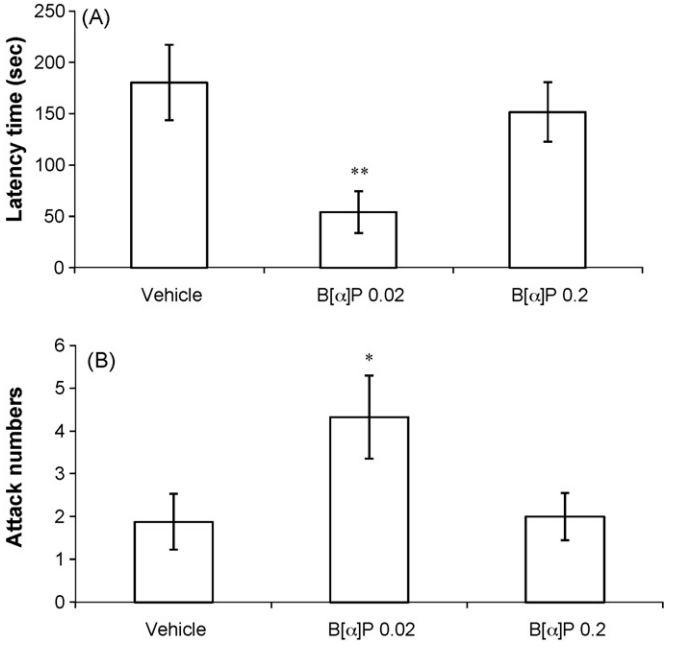

Fig. 1. Effects of subacute oral exposure to B[α]P (0.02 and 0.2 mg/kg) on the latency time of the first attack (A) and the number of attacks (B) during resident-intruder test designed to measure aggressive behaviour. B[α]P was given to mice for 3 weeks ($n = 9$). Data are reported as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$.