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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).

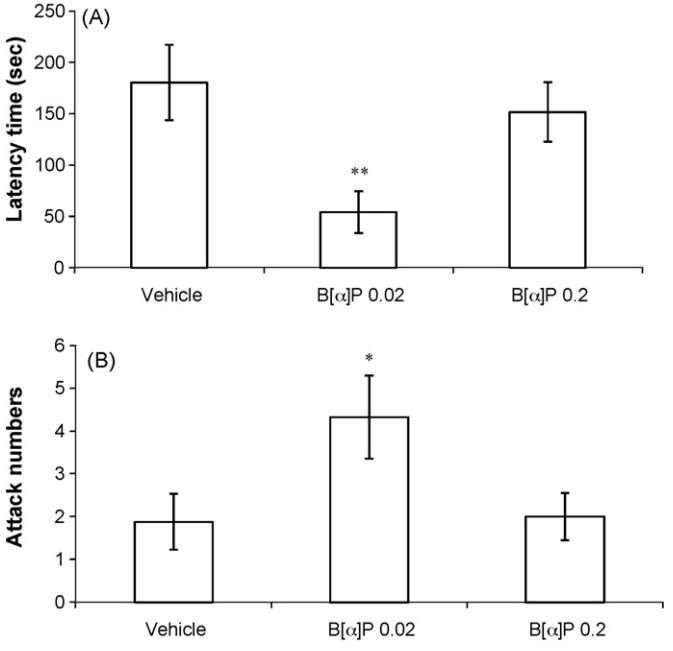

Fig. 1. Effects of subacute oral exposure to B[α]P (0.02 and 0.2 mg/kg) on the latency time of the first attack (A) and the number of attacks (B) during resident-intruder test designed to measure aggressive behaviour. B[α]P was given to mice for 3 weeks ($n = 9$). Data are reported as mean ± SEM. $^{*}p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

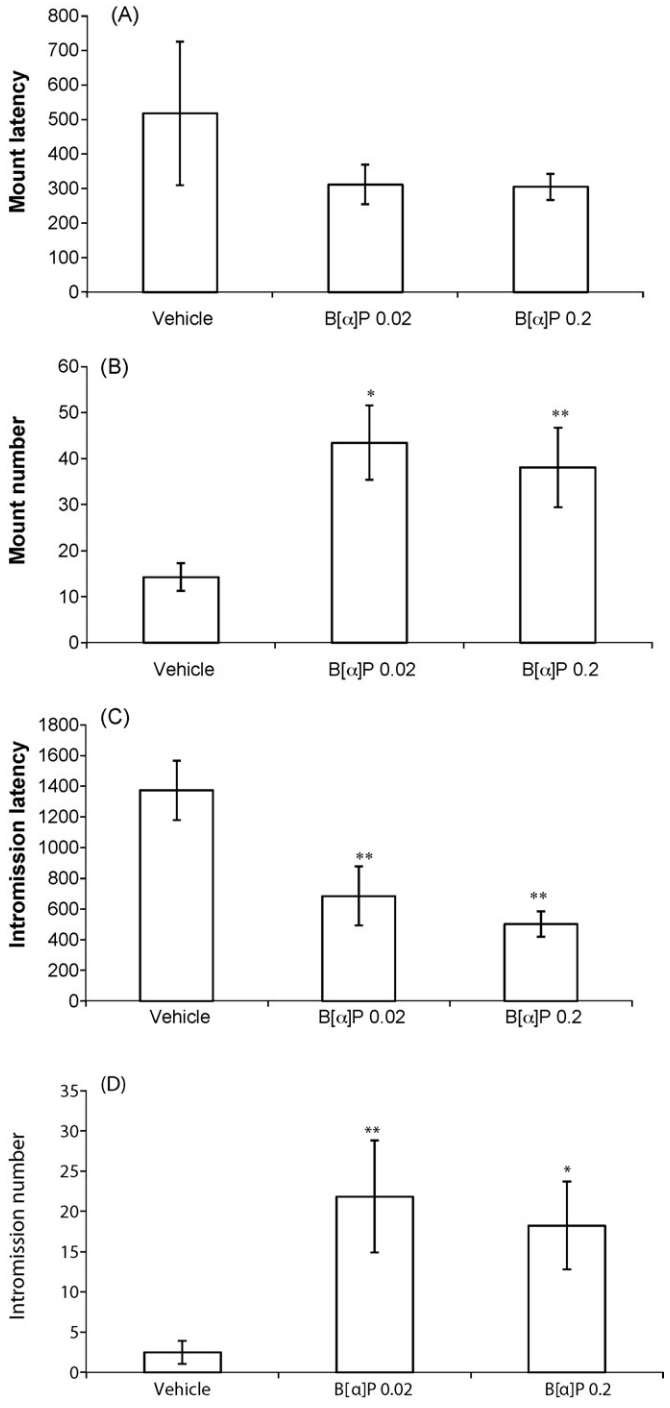

Fig. 2. Effects of subacute oral exposure to B[α]P (0.02 and 0.2 mg/kg) on the latency to the first mount (A), mounting (B), intromission latency (C) and intromission frequency (D) during sexual behavioural test. B[α]P was given to mice for 4 weeks ($n = 8$). Data are reported as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$.

3.2. Subacute effect of B[α]P on male mice copulatory behaviour in sexual behavioural test

4 weeks of treatment of male mice with B[α]P induced a significant increase in mounting ($p < 0.05$), intromission latency ($p < 0.01$) and intromission frequency ($p < 0.01$) in B[α]P 0.02 mg/kg-treated mice compared to control mice (Fig. 2). In addition, significant differences were also found between B[α]P 0.2 mg/kg-treated mice and control mice with respect to the mounting ($p < 0.01$), intromission latency ($p < 0.01$) and intromission frequency ($p < 0.05$) (Fig. 2). No significant differences were found between B[α]P (0.02–0.2) mg/kg-treated mice and control mice with respect to the mount latency ($p > 0.05$) (Fig. 2).

4. Discussion

The present study was conducted to examine the harmful effects of subacute oral exposure to B[α]P on both aggressive and sexual behaviours in Swiss albino male mice. The doses of B[α]P (0.02 and 0.2 mg/kg) used in this study can be potentially ingested by human in the ambient environment such as the case of individuals with heavy occupational exposure to B[α]P or heavy consumers of smoked or grilled meat and fish among others [37,38]. It is worth pointing out that some adverse effects of B[α]P on behaviour have been noted at doses higher than the levels found in the ambient environment [5,13,14,25,27]. Here, we found that prolonged exposure to B[α]P affects aggressive behaviour in a dose-independent manner in the resident-intruder test. The latency time of the first attack has been significantly shortened in B[α]P 0.02 mg/kg-treated male mice in comparison to control mice. Furthermore, the number of attacks has significantly increased in B[α]P 0.02 mg/kg-treated male mice than control. Our results showed therefore that subacute oral exposure (3 weeks) to B[α]P significantly increases aggressive behaviour in male mice. The fact that only prolonged exposure to B[α]P at the dose 0.02 mg/kg significantly increases aggressiveness in male mice can be explained by that this dose could not induce the biochemical change in the same manner than at the dose 0.2 mg/kg. The effect of B[α]P on central nervous system signaling pathways is complex and not well defined. For instance, in another study (unpublished results), we have found that subacute oral exposure to B[α]P at the dose 0.02 mg/kg significantly increased the gene expression levels of the two brain receptors for serotonin ($5HT_{1A}$) and noradrenalin (alpha-1D adrenergic: $ADRA_{1D}$) in female adult mice. Noteworthy, higher doses of B[α]P (0.2 and 20 mg/kg) did not produce a significant response on the expression of $5HT_{1A}$ and $ADRA_{1D}$ mRNA. Recently, we showed that lactational exposure to B[α]P impacts the serotoninergic system, however, by significantly decreasing the gene expression of $5HT_{1A}$ in pup mice exposed to B[α]P (2–20 mg/kg) through lactation in comparison to controls [26]. No significant changes in the gene expression of $ADRA_{1D}$ between pups whose mothers were exposed to B[α]P and vehicle alone were found [26]. Stephanou et al. [8] reported that both acute and subacute exposure of adult rats to B[α]P caused a decrease in catecholamine levels in several brain areas including the striatum, hypothalamus and midbrain. However, an increase in serotonin levels has been observed in the midbrain and cortex. The increase in the brain serotonin levels has also been noted by Jayasekara et al. [9] after a subacute exposure of adult mice to B[α]P. In contrast, these authors found also an increase in catecholamine levels in the striatum and hypothalamus [9]. Grova et al. [13,25] showed that B[α]P significantly up-regulates the expression of NMDA receptor subunit 1 (NR1) in the hippocampus of adult mice, however, Wormley et al. [39] showed that B[α]P down-regulates NR1 subunit protein in the hippocampus of B[α]P exposed F1 generation rats. It is interesting to note that Grova et al. [13,25] also found a negative dose–response relationship of B[α]P in mice. These authors have been reported that B[α]P only at low doses (0.02–0.2 mg/kg) significantly provoked amnesic action. However, at high doses (20–200 mg/kg), B[α]P significantly decreased anxiety-related behaviour without affecting working memory in female mice in the Y-maze spontaneous alternation. Moreover, in young mice whose mothers were orally exposed to B[α]P, we showed that the lower dose (2 mg/kg) was active, but the higher dose (20 mg/kg) was not on immediate working memory performance in the Y-maze test [26].

In addition, this study also showed that B[α]P affects copulatory behaviour in male mice. Indeed, after 4 weeks of exposure to this xenobiotic, 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, which are markers for the consummatory aspects of copulatory behaviour [35], than control mice. Our results suggested therefore that B[α]P affects the consummatory aspects of sexual behaviour. However, there was no effect of B[α]P on the latency to the first mount, an indicator of appetitive aspects and sexual motivation in copulatory behaviour [35], suggesting that appetitive aspects were not altered in B[α]P-treated mice. Considering that male mice rarely focus their aggression towards female [40], it is interesting to note here that we have also observed that B[α]P-treated male mice have been more aggressive toward female mice after sexual act than control mice (results not shown). Despite that B[α]P increases sexual behaviour of male mice, we cannot conclude that this neurotoxic pollutant has advantage of sexual function since B[α]P has been shown to alter the monoaminergic system [8,9,26] and causes endocrine dysregulation [7] *via* toxic effect. In this sense, it has also been shown that subchronic inhalation exposure (60 days) to B[α]P alters sexual function in rats [7] by impairing testicular endocrine and exocrine function, by increasing the rate of spermatozoa with abnormal morphologies and by reducing sperm motility which is positively correlated with fertilization of oocytes [41] and pregnancy rates [42]. Other animal studies have also demonstrated that PAHs including B[α]P alters male reproductive function [43,44]. Additionally, Selevan et al. [45] and Sram et al. [46] have established a direct relationship between exposure to PAHs and the poor semen quality in young men in Czech Republic exposed to air highly polluted with PAHs.

In this study, although the precise mechanisms underlying the effects of B[α]P on the sexual function and aggressiveness is yet unclear, it seems to be plausible that the observed effects of this neurotoxic compound result from its negative impacts on the central monoaminergic neurotransmitter activities including serotonin which play a central role in the regulation of aggression, sexual behaviour among others [35,40,47]. Furthermore, the dysfunction of endocrinological system in B[α]P-treated mice cannot rule out in view of the evident role of this system in sexual behaviour and aggressiveness [48–51]. The negative effects of prolonged exposure to B[α]P on sexual function and aggressiveness in B[α]P-treated mice may be specific, since B[α]P (0.02–0.2 mg/kg) does not affect anxiety levels as we have revealed it by using the light/dark choice test (results not shown).

In conclusion, subacute oral exposure to B[α]P increases aggressiveness in male mice. This harmful effect was also associated with sexual behaviour disturbances in these animals. Indeed, subacute oral exposure to B[α]P significantly increases the consummatory aspects of sexual behaviour in male mice. Our findings confirm previously hypothesis [8] supposing that prolonged exposure to B[α]P may lead to behavioural disturbances. This study gives evidence that B[α]P, which is an environmental pollutant, alters both aggressive and sexual behaviours. Our results raise the question of the effect and/or the role of other environmental toxins on aggressiveness and copulatory behaviour.

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