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Early post-natal neuroactive steroid manipulation modulates ondansetron effects on initial periods of alcohol consumption in rats

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ABSTRACT

Neuroactive steroids (NS) such as allopregnanolone are crucial for brain development and adult behaviour. Early post-natal alterations of NS by administering finasteride induce a decrease in the sensitivity to stimulant effects of low alcohol doses, an increase in alcohol consumption, and a decrease in ventrostriatal dopamine and serotonin levels. The aim of the present study is to observe if the effects of the 5HT3 receptor antagonist ondansetron on initial alcohol consumption are modulated by post-natal NS manipulation. For this purpose, allopregnanolone, finasteride, or vehicle was injected from day 5 to 9. In adulthood, a novel object preference test was carried out in order to detect a possible novelty-seeking pattern in our animals, which has been related to vulnerability to drug abuse. The subjects then had access to two bottles (alcohol or control solutions) one hour daily for two consecutive weeks. Ondansetron (0.01 mg/kg, 0.1 mg/kg or vehicle) was administered before the hour of consumption in the initial phase (days 1, 2, 3) of the procedure, and after prolonged alcohol intake (days 11, 12, 13). Results indicated that finasteride animals showed a higher preference to explore the new object, as well as a higher alcohol consumption than the rest of the groups. Moreover, 0.1 mg/kg of ondansetron decreased alcohol consumption, but only in the post-natal finasteride group, suggesting a possible increase in 5HT3 receptor sensitivity in these animals. In conclusion, NS manipulation in crucial stages of development, such as early post-natal periods, seems to play an important role on the effects of ondansetron on alcohol intake and in the vulnerability to develop drug use or abuse.

1. Introduction

Neuroactive steroids (NS) can alter neuronal activity regardless of its origin (adrenal, gonadal, or cerebral) [1, 2]. One of the most important mechanisms of action of the NS in the central nervous system is the allosteric modulation of the ionotropic receptors, such as GABAA, 5HT3 serotonin, or nicotinic acetylcholine receptors [3–5].

It has been observed that NS, acting as positive or negative GABAA modulators, are essential for brain development. In this way, it has been described that an increase in the positive GABAA modulator allopregnanolone (AlloP) during post-natal periods affects the thalamic-cortical pathways development [6]. Furthermore, the administration of negative GABAA modulators, such as pregnenolone or dehydroepiandrosterone, in early stages of development changes the activity of dopamine (DA) in the striatum [7]. On the other hand, finasteride, a 5α-reductase inhibitor (enzyme involved in the synthesis of AlloP, tetrahydrodeoxycorticosterone, and dihydrotestosterone [8]), has been used as a tool to manipulate endogenous levels of NS that are positive modulators of GABAA receptors. Thus, it has been documented that early post-natal administrations of finasteride affect the expression of GABAA alpha4 and delta receptor subunits in the hippocampus [9].

Furthermore, several studies have reported that post-natal NS alterations can modify adult behaviour (for a review see [10]). For example, it has been observed that early post-natal administration of AlloP increased locomotor activity in a novel environment, and reduced the anxiolytic-like effects of the benzodiazepine lorazepam [11], decreased anxiety in the elevated plus-maze (EPM) test [12, 13], and increased activity induced by amphetamine administration [14]. A number of these behavioural traits related to AlloP administration (i.e. increase in novelty exploration and anxiety decrease) have been linked to a sensation/novelty seeking pattern, which at the same time has been related to a vulnerability to use and abuse of several drugs, including alcohol [15–17]. In previous experiments it was observed that post-natal NS manipulation by means of finasteride administration increased voluntary consumption of an ethanol (EtOH) solution in a 1 h/day limited access procedure [18, 19], decreased ventrostriatal DA and serotonin levels [18], and decreased DA release in the nucleus accumbens after EtOH or food presentation [19]. These last results

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suggest a possible hypo-dopaminergic activity related to early post-natal finasteride administration that could have induced an increase in alcohol consumption. Moreover, it has been documented that DA inhibition seems to produce a decrease in the sensitivity to EtOH stimulating effects [20]. Consistent with this last data, we have recently reported that early post-natal finasteride administration decreased locomotor activity induced by a low dose of EtOH [21].

Interestingly, it has been reported that AlloP brain levels increase after the administration of EtOH [22–25], although there are sexual [26] and species [27] differences in this effect. Furthermore, part of the EtOH behavioural effects may be related to the increase in endogenous AlloP levels. Thus, it has been found that a pre-treatment with finasteride attenuates the anxiolytic action of EtOH [28]. Moreover, the development of tolerance to the depressing effects of EtOH is linked to a reduction in AlloP production [23, 29]. On the other hand, the administration of AlloP also affects the EtOH consumption, producing an increase in intake in non-dependent rats with low consumption levels [23, 30]. Furthermore, systemic AlloP administration decreases alcohol intake in dependent rats with high consumption levels [23], including when AlloP is administered into the dorsal hippocampus [31]. In this way, it has been observed, in mice, that low doses of AlloP (i.p. and i.c.v.) increase EtOH intake, whereas high doses of AlloP decrease it in limited access EtOH drinking [32], and in the EtOH operant self-administration paradigm [33]. Moreover, it has been observed that intrahippocampal AlloP administration reduces EtOH withdrawal symptoms, such as audiogenic seizures and body rigidity [34], while finasteride increases their severity [35].

Both alcohol and AlloP modulate 5HT3 receptors. Whilst alcohol acts as a positive allosteric modulator of this receptor [36], AlloP acts as negative modulator [5]. Of importance, is that 5HT3 receptor seems to be related to the alcohol rewarding properties throughout the modulation of DA release in the nucleus accumbens [37–40]. For this reason, it has been proposed that differences in serotonergic signalling may participate in alcoholism vulnerability [41, 42]. The systemic administration of 5HT3 receptor antagonists has been shown to effectively reduce alcohol intake in rats in free-choice conditions [43–46]. However, although their efficacy is variable depending on drinking conditions after stabilization of ethanol intake [45], there are no studies focusing on their possible effects in the initial periods of alcohol intake, which can be important for the later development of drug abuse. On the other hand, it has been hypothesised that 5HT3 receptors are potential key-regulators of network formation and function in the developing brain [47]. Given that NS are modulators of 5HT3 receptors at micromolar concentrations [5], its maturation could be affected by post-natal NS manipulation, and consequently, alcohol rewarding properties could be modified. It should be mentioned that previous experiments carried out in our laboratory reported hippocampal levels of AlloP in the micromolar range in PN5 [48] and PN9 [49] after post-natal injections of 20 mg/kg of AlloP. Moreover, although finasteride administration during the neonatal period affects the expression of some GABAA receptors in adult age [9], it is not known how it may affect neuroactive steroids levels in adult animals. Thus, it is important to consider that finasteride, by inhibiting the 5alpha-reductase enzyme, affects levels of all the 5-alpha-reduced neuroactive steroids, not only those of AlloP.

The aim of the present study is to determine whether the effects of the pharmacological antagonism of the serotonin 5HT3 receptor on initial alcohol intake would be affected by post-natal NS manipulation. For this purpose, AlloP, finasteride or vehicle was administered to newborn male Wistar rats. In adulthood, the animals were subjected to one hour per day of EtOH intake for two weeks, and were administered with the 5HT3 antagonist, ondansetron, on days 1-2-3 and 11-12-13 of alcohol intake days. Moreover, as novelty seeking (preference for a novel environment in a free-choice situation) has been shown to be a predictor of substance use disorder outcomes in animals and humans [50–52], including the risk for the initiation of drug use [53], we decided to investigate the possible effects of post-natal treatments on novelty-seeking that could be linked to posterior alcohol use/abuse vulnerability. As used in previous works [54–56], the novelty object preference (NOP) test was chosen as a behavioural test of free-choice novelty-seeking. The NOP task is based on rodents' natural tendency to explore novel objects and provides subjects with the opportunity to choose between a novel and a familiar stimulus [57, 58]. Although NOP has been frequently used for memory testing, the NOP task also allows researchers to investigate the mechanisms involved in novelty seeking [57–59]. For instance, it has been suggested that acute stress decreases NOP probably by inducing an emotional arousing state which motivates novelty avoidance, and not though a memory retrieval impairment [60]. Moreover, spontaneous high impulsivity rats, which have a high vulnerability to cocaine abuse, showed a general preference for novel objects or contexts in comparison to familiar ones [61].

In the present experiment, we hypothesised that early post-natal NS alteration will affect novelty-seeking behaviour, as well as the effects of ondansetron on EtOH intake, and will be different depending on post-natal NS manipulation, suggesting an altered 5HT3 receptor function.

2. Materials and methods

2.1. Animals

106 male Wistar rats were bred in the Laboratory of Psychobiology (Autonomous University of Barcelona), and housed in a temperature-controlled animal room (22–24 °C) on 12 h light/dark cycle (lights on at 8:00H) on a food and water ad libitum. The day of birth (post-natal day (PN) 0) was strictly controlled and the litters were reduced to 10 pups. In order to avoid any effect of cohorts, each litter of the same parents was assigned to different post-natal treatments (AlloP, finasteride or vehicle) (detailed below). After weaning (PN 21) males were separated into groups of siblings with a maximum of four and a minimum of two animals per cage (46.5 × 21.5 × 19.5 cm) and females were sacrificed. At PN75 the animals were housed individually, two days before starting alcohol consumption procedure. No visible animal health alterations were detected during development as a consequence of the post-natal treatments or during behavioural evaluations. All animals were obtained, housed, and sacrificed in accordance with the protocol approved by the Committee of the Autonomous University of Barcelona for Care and Use of Experimental Animals and the Department of Environment from Generalitat de Catalunya. This protocol follows the guidelines approved by the European Council Directive (2010/63/EU).

2.2. Early post-natal treatment

Pups were injected (s.c.) with AlloP (3α-hydroxy-5α-pregnan-20-one, 20 mg/kg), finasteride (N-tert-Butyl-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide, 50 mg/kg) or vehicle (10% 2-hydroxypropyl-β-cyclodextrin dissolved in distilled water), once a day from the post-natal day 5 to 9 (PN5-PN9). AlloP and finasteride were dissolved in distilled water and 10% 2-hydroxypropyl-β-cyclodextrin. The doses were chosen based on previous experiments which show that they were effective in changing behaviour in adulthood (for a review see [10]). For injections, each litter was removed from progenitors and returned to its cage in < 12 min. The chemicals were obtained from Sigma (Deisenhofen, Germany).

2.3. Adult treatment

Subjects were administered (i.p.) with the 5HT3 antagonist ondansetron (ondansetron hydrochloride dehydrate (C18H19N3O·HCl·2H2O) obtained from Sigma), and were randomly divided into three groups: 0.01 mg/kg, 0.1 mg/kg or vehicle (saline solution (0.9% NaCl)). The doses were chosen as they were effective to decrease alcohol intake [62] or to reduce DA efflux enhanced by morphine and haloperidol [63] in previous works. The administration took place 30 min before solutions presentation on days 1, 2, 3 and 11, 12, 13, at the beginning of the initial and final phases of alcohol consumption (see section 2.5.).