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UTILITY OF NEUROSTEROIDS IN RESTORATION OF EFFECTS OF ETHANOL: A CRITICAL EVALUATION

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Abstract

The article will focus on various physiological conditions linked to the activity of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes, the consequences of chronic ethanol contact, as well as behavioural consequences for how neurosteroids affect sensitivity to ethanol. The alteration of ethanol impacts by neuroactive steroids has so far been mostly focused on pathways involving -aminobutyric acid receptors. Although NMDA receptor pathways are becoming more prominent in the literature, it would be appropriate to discuss this complicated data individually. In order to highlight the divergent neuroactive steroid pharmacology, receptor mechanisms are highlighted in this review along with a quick discussion of some NMDA receptor processes. Overall, the data imply that neurosteroids are essentially universal inhibitory neurotransmission modulators. According to certain behavioural studies, neurosteroids appear to impact sensitivity to ethanol in particular brain regions. This effect may be connected to how well and effectively ethanol can stimulate the release of receptors and raise neurosteroid concentrations. Although some investigations have revealed a direct interaction between ethanol and neuroactive steroids at similar receptor binding sites, this idea is still debatable. The precise method through which neuroactive steroids could modify the effects of ethanol in particular behavioural tasks is still impossible to pinpoint.

Keywords – Neurosteroids, Restoration, Ethanol, alcohol withdrawal, reinstatement

Introduction

Abuse and dependence related to alcoholism are a global issue from a social and medical perspective. A habit of obsessive drinking and a physiological dependence on alcohol are characteristics of the condition known as alcohol dependence. A well-defined group of

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symptoms known as acute alcohol withdrawal (AW) is produced when long-term alcohol usage is abruptly reduced or completely stopped. Chronic alcoholism has negative effects on the central nervous system (CNS), including brain atrophy and/or malfunction; the lifetime dose of ethanol consumed is connected to these brain impairments.

When an animal initiates a particular behavioural reaction after learning it and then letting it go, a process known as reinstatement, it is possible to detect relapse in laboratory animals. Re-acquisition and spontaneous recovery are two additional techniques that can be employed in the CPP to measure relapse in addition to reinstatement. Recent research on relapse into drug usage in laboratory animals uses the extinction reinstatement variation of this model. In this paradigm, animals are initially taught to learn a behaviour before going through a process of extinction. Animals are then exposed to various cues in order to assess how well they prompt the reinstatement of the previously learned and suppressed behaviour.

The reinstatement model comes in two main forms: one based on the operant self-administration procedure, where the learned, suppressed, and reinstated behaviour is typically pressing a lever, and the other based on the classical conditioned place preference procedure, where the learned, suppressed, and reinstated behaviour is CPP.

Although the majority of the results from the CPP model of reinstatement are consistent with those from self-administration research, several inconsistencies have been found. These disparities are assumed to be the result of either different methodologies or various response criteria that were employed to measure reinstatement. It should also be remembered that the CPP and self-administration paradigms assess various aspects of reward and, hence, various aspects of relapse and addictive behaviour. While CPP, which is used to study the conditioned rewarding effects of drugs and other stimuli, involves a Pavlovian conditioning, models cue-elicited drug-taking behaviour, and evaluates the incentive value of drug-associated cues for maintaining addictive behaviour, self-administration involves an operant conditioning, models drug-taking behaviour, and assesses the primary rewarding properties of drugs. While the reinstatement of CPP entails the return of the approach behaviour to a drug-

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associated setting, the reinstatement of self-administration after extinction necessitates the restoration of a concrete operant response.

It is challenging to judge the validity of the CPP version of the reinstatement model as a technique for identifying medications with therapeutic potential in people because only a small number of substances have been examined to date. Only naloxone and acamprosate have been evaluated in the CPP reinstatement model so far of all the medications that have been FDA-approved for treating misuse.

Self-administration paradigms unquestionably offer a face-relevant model, and it may be claimed that they more closely mirror the human state. Contrarily, despite the fact that CPP paradigms' simplicity restricts the variables that may be investigated, CPP assays for the investigation of reward and reliance have grown in popularity as a result of their ease of use. The most significant finding is that the medicines and brain pathways that are suggested to play a role in reward and relapse based on self-administration assays and those suggested to play a role based on CPP test are in good agreement. The benefit of the CPP reinstatement model is that because the dependent measure is not operant-based response, nonspecific motor effects of pharmacological treatments may be less likely to affect behaviour. Additionally, it can often be accomplished more quickly, more cheaply, and methodologically (by a single drug-context pairing), and it is sensitive to quite low drug dosages.

Review of literature

By using a sex-specific mechanism, the neurosteroid 3alphaDIOL modifies place preference when injected into the basolateral amygdala (Pérez-Acevedo et al., 2016).

Systemic 3alpha-diol (1.0 mg/kg) prior to exposure to the non-preferred side of a CPP chamber significantly increased preference for the non-preferred side of the chamber compared to baseline preference and home cage controls. This was in contrast to baseline preference and home cage controls. Additionally, compared to control groups, the administration of testosterone, dihydrotestosterone, or 3alpha-diol increased levels of these androgens, reduced ARs (reduced intrahypothalamic AR and seminal vesicle weight), and

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improved GBR function (reduced GABA-stimulated chloride influx in cortical synaptoneurosomes and muscimol binding in the hippocampus). The concentrations of 3alpha-diol were raised in the Nucleus Accumbens (NA) following systemic treatment of 3alpha-diol that improved CPP. In addition to producing a CPP in comparison to baseline preference and vehicle controls, central implants of testosterone, dihydrotestosterone, or 3alpha-diol to the NA also did so (Rosellini et al., 2021).

Pregnenolone sulfate-induced seizures were characterised by head jerks, rearing and falling, severe forelimb and hindlimb clonus, opisthotonos, and explosive running at doses of 50 to 150 nmol. With time, the seizures become worse and more frequent, leading to status epilepticus, tonic hindlimb extension, and death. The convulsant potencies of systemically administered pentylenetetrazol (30–50 mg/kg) and NMDA (50–100 mg/kg) were dramatically boosted by a subconvulsant dosage of pregnenolone sulphate (50 nmol). Pregnenolone sulphate, when given systemically in dosages as high as 100 mg/kg, did not cause seizures or change the convulsant potencies of pentylenetetrazol and NMDA (Kokate et al., 2019).

Exposure to NMDA altered pregnenolone and pregnenolone sulphate production in the isolated retina. (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] inhibited NMDA-stimulated neurosteroid production.MK-801 and 3(2-carboxypiperazine-4-yl)propyl-1-phosphonic acid (CPP), which depended on extracellular calcium and were generated by glutamate, respectively (Guarneri et al., 2018).

Pregnenolone sulphate, a neurosteroid, has recently been demonstrated to positively modify NMDA receptors and to improve memory in mice. In a step-through passive avoidance test, it improved retention and decreased impairments brought on by a competitive NMDA receptor antagonist, 3-((+/-)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) (Mathis C et al., 2014).

In isolated retina, pregnenolone sulphate induces and amplifies acute excitotoxicity by modulating NMDA receptors (Guarneri et al., 2018).

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According to Font et al. (2008), the brain's catalase-H(2)O(2) system supports the role of centrally generated acetaldehyde in the creation of ethanol-induced pleasant affective memories as well as the acquisition of affective-dependent learning.

Using the conditioned place preference paradigm, the GABA A-receptor agonist neuroactive steroid 3 alpha,5 alpha-P(3 alpha-hydroxy-5 alpha-pregnan-20-one), an endogenous regulator of GABA A- receptor function, has rewarding qualities (Finn 1997 et al., 2017).

Only in rats with a history of ethanol dependency did a stimulus that was conditioned to foot-shock stress result in the resumption of ethanol seeking behaviour and an increase in ethanol seeking response (Liu et al., 2002).

Foot shock stress and alcohol priming injections resulted in the resumption of alcohol seeking behaviour (Li et al., 1998).

According to Gass et al. (2007), pharmacological stressors, ethanol priming, or ethanol-related stimuli can restore ethanol seeking behaviour.

Objective of the study

To investigate the role of neurosteroids in ethanol-induced locomotor sensitization, ethanol-induced conditional place preferences, and ethanol-induced conditional place preferences that have been reinstated.

Research Methodology

The present studies used adult male Swiss albino mice (24–30 g) that were born and raised in the animal house of the Agnihotri College of Pharmacy, Wardha, from a stock that was initially obtained from Wockhardt Research Centre, Aurangabad, India. Animals were kept in groups of four in opaque polypropylene cages (282114 cm) with a 12:12 h light/dark cycle (light cycle: 0800–2000 h), at a temperature of 23.2 °C, with free access to a standard mouse feed and tap water. At the start of every trial, animals were naive to drug use and

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experimentation. Each experiment had six to eight animals in each group. To reduce (if any) circadian impacts, behavioural investigations were conducted between 9:00 and 14:00. In a quiet room, testing was done in a counterbalanced order with respect to the treatment conditions. The Institutional Animal Ethics Committee (IAEC), established by the Ministry of Environment and Forests, Government of India, New Delhi, India, to oversee and govern the use of experimental animals, gave its approval to each experiment.

Drugs and solutions

Progesterone (4-pregnene-3,20-dione; Susten 100; Sunpharmaceutical Ind. Ltd., Gujarat, India) was dissolved in a medium containing Finasteride (Dr. Reddy's Laboratories, Hyderabad, India) and a 1:2:7 mix of Tween 80, DMSO, and saline (Umathe et al., 2009). Dehydroepiandrosterone sulphate (DHES) was dissolved in a 20% solution of 2-hydroxypropyl--cyclodextrin, and ethanol (Changshu Yangyuan Chemical, China) was dissolved in 0.9% saline (Matthew et al., 2005). Based on our early research, progesterone and DHES were given at a dose of 5 mg/kg intravenously, and FIN at a dose of 50 mg/kg intravenously. As these levels had previously been proven effective for behavioural tests, ethanol was given at a dose of 0.5-2 g/kg; intravenously in a volume of 10 ml/kg body weight. (Risinger and Oakes, 1996; Maurice et al., 2003; Font et al., 2006, 2008; Bhutada et al., 2010a).

Data Analysis and interpretation

Effect of ethanol dose response on the Conditioned Place Preference test

Figure 1 depicts the ethanol's dose-dependent influence on the emergence of conditioned place preference (CPP). According to a one-way ANOVA, the ethanol treatment had a significant impact on how long subjects spent in the ethanol-conditioned chamber during the CPP-I test [F (4, 35) = 17.79, P0.0001]. A post hoc analysis also showed that higher dosages of ethanol (1.5 and 2.0 g/kg, i.p.) significantly lengthened the duration spent in the ethanol-paired chamber relative to the vehicle control (P0.05 and P0.001, respectively), whereas lower doses (0.5 and 1.0 g/kg) had no significant impact on this parameter.

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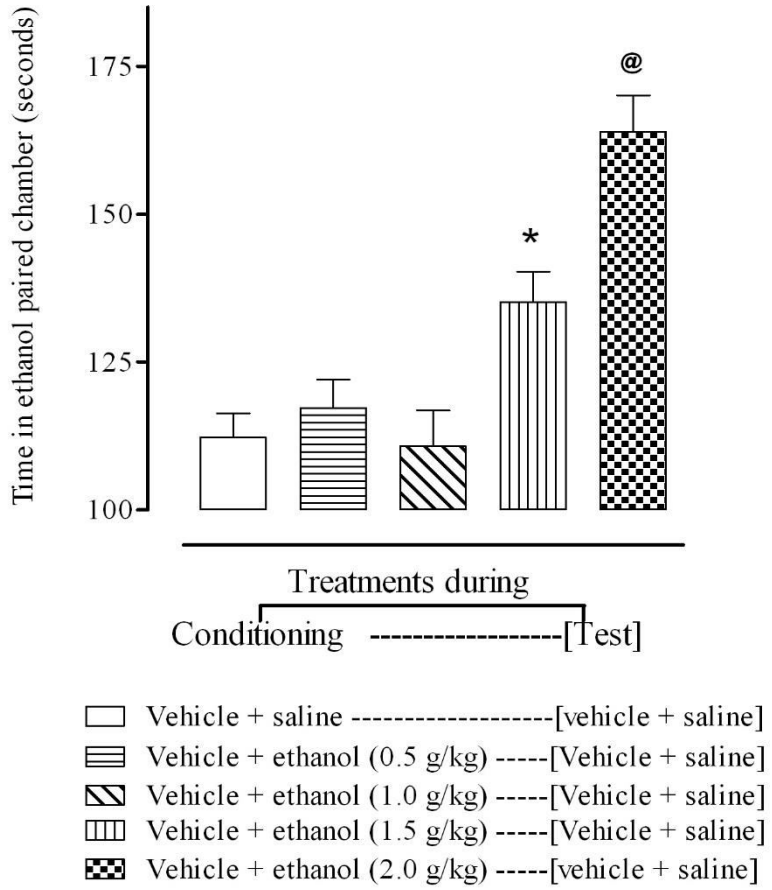

Fig.1. Effect of ethanol dose response on the Conditioned Place Preference test.

Each value is the mean S.E.M. (n=7-8), and each value. In comparison to [(vehicle + saline in conditioning) and [(vehicle + saline in testing)], $P<0.05$, $P<0.001$. Tukey's multiple comparison test is used after a one-way ANOVA.

Progesterone, FIN, and DHES's impact on the development of ethanol-induced Conditioned Place Preference

One-way When compared to the vehicle-pretreated group, an ANOVA showed that mice pretreated with progesterone (5 mg/kg, i.p.) spent significantly more time on day 10 in the ethanol-paired chamber during the CPP-I test on days 2, 4, 6, and 8 (during the development of ethanol-CPP) [$F(4, 29) = 19.94$, $P<0.0001$] (Fig. 2). Further analysis using a post hoc test showed that pretreatment with progesterone (5 mg/kg, i.p.) before ethanol (0.5 g/kg)

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significantly increased the amount of time spent in the ethanol-paired compartment (P<0.001).

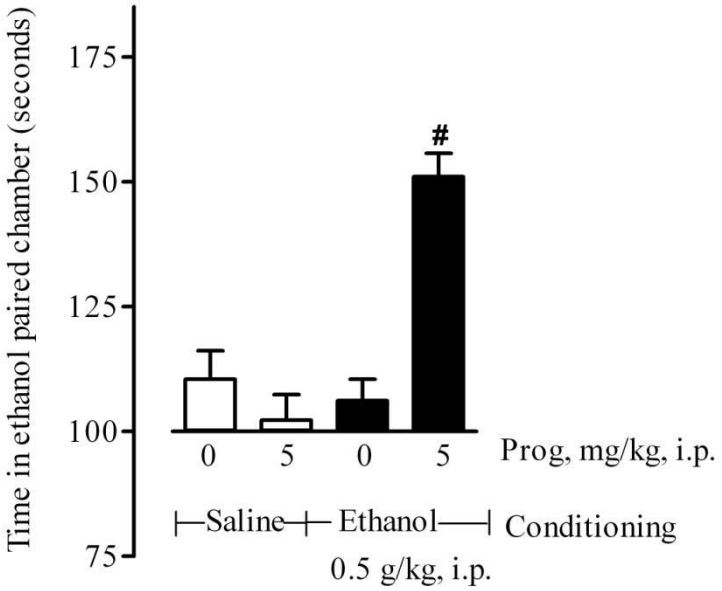

Fig.2. Progesterone's impact on the development of ethanol-induced conditional place preference.

Each value is the mean S.E.M. (n=7-8), and each value. P<0.001 in the conditioning group vs. vehicle + ethanol (0.5 g/kg). The results of a one-way ANOVA showed that mice given FIN (50 mg/kg, i.p.) or DHES (5 mg/kg, i.p.), 10 min before each ethanol (2.0 g/kg, i.p.) injection on days 2, 4, 6, and 8 (during the development of ethanol-CPP), spent significantly less time in the ethanol-paired chamber on day 10 during the CPP-I test [F(4,30) = 14.51, (Fig.10 and 11). The acquisition of the ethanol-CPP response was considerably decreased by FIN (50 mg/kg, i.p.) and DHES (5 mg/kg, i.p.), according to a post hoc analysis (P<0.001 and P<0.001, respectively). In addition, post hoc analysis showed that sub-chronic administration of FIN (50 mg/kg, i.p.) and DHES (5 mg/kg, i.p.) in the saline-conditioned group did not exhibit any preference for either chamber.

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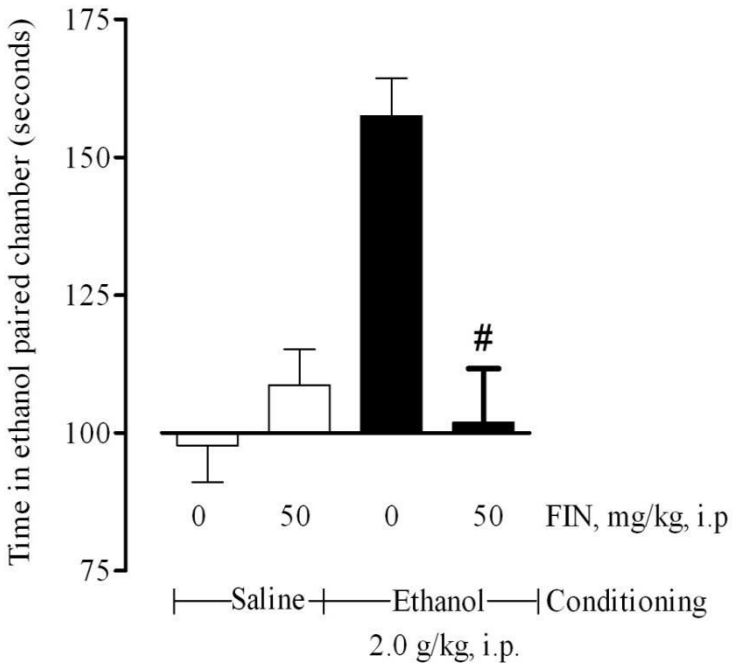

Fig.3. Alcohol-induced conditional place preference acquisition and the neurosteroid biosynthesis inhibitor FIN.

(n=7-8); P<0.001 vs. (vehicle + ethanol in conditioning) treated group (one-way ANOVA followed by Tukey's multiple comparison test); each value represents the mean S.E.M.

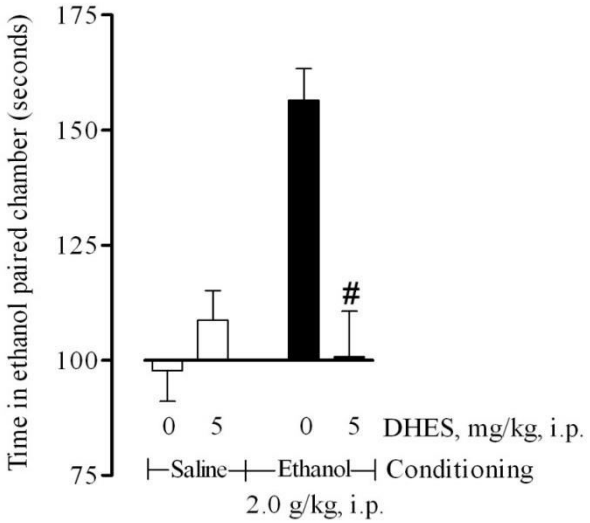

Fig.4. Effect of ethanol-induced conditional place preference acquisition on the neurosteroid antagonist DHES.

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Each value is the mean S.E.M. (n=7-8), and each value. P<0.001 versus the group that received treatment (vehicle + ethanol in conditioning) (one-way ANOVA followed by Tukey's multiple comparison test).

Conclusion

Researchers showed that ethanol and stress-induced CPP are reestablished by neurosteroids agonist, whereas ethanol-induced locomotor sensitization and stress-induced conditioned place preference behaviour are attenuated by neurosteroids antagonist. The findings of this investigation provide credence to the idea that neurosteroids play a role in the reinstatement of ethanol and the motivational effects in experimental animals. Studies on many animal species should be conducted to verify these findings.

To corroborate the role of Neurosteroids that has been observed, blood and brain ethanol concentrations should be evaluated following ethanol abstinence. It is important to do immunocytochemical research to confirm the function of neurosteroids in certain brain regions. To further support the function of Neurosteroids in ethanol-induced reinstatement effects and distinct acute and chronic effects of ethanol, studies on these effects should be conducted. Research on the various acute and long-term impacts of ethanol-induced reinstatement and motivational effects should be conducted to determine additional biochemical parameters including catalase and superoxide dibismutase (SOD).

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