

INTERACTION BETWEEN CHOLINERGIC AND OPIOID SYSTEMS IN RELAPSE TO ALCOHOL DEPENDENCE

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Received: 23 Oct 2014 Revised and Accepted: 15 Nov 2014

ABSTRACT

Objective: To confirm the interaction between cholinergic and opioid systems in alcohol dependence using an animal model.

Methods: Experiments were conducted using Conditioned Place Preference (CPP) paradigm. Mice were conditioned with alcohol, nicotine and combination of both. They were then subjected to postconditioning test, in which their preference scores were measured. Following a period of drug abstinence, they were reinstated by morphine at doses of 5, 10, 20 and 40 mg/kg BW to induce relapse. Acetylcholinesterase (AChE) activity measurements were performed at the end of the behavioral tests using Ellman's method.

Results: Priming dose of morphine of 10 mg/kg, 20 mg/kg and 40 mg/kg BW increased significantly the preference score during relapse to alcohol compared with the score in post conditioning test. AChE activity in animal at the time of relapse was significantly different compared to saline treated group. The highest enzyme activity was shown after priming dose of 20 mg/kg BW in group conditioned with alcohol and nicotine. There were no significant differences between the activity of AChE in groups receiving 5 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg BW of morphine challenge.

Conclusion: Result of the present study indicated that morphine challenge in alcohol dependent animals during drug abstinence induces relapse to alcohol dependence. This is accompanied by increased AChE activity suggesting cholinergic-opioid system interaction.

Keywords: Alcohol, Morphine, Conditioned place preference, Relapse, Acetylcholinesterase.

INTRODUCTION

Alcohol consumption and its problems differ from one country to another. Approximately 4% of deaths worldwide were due alcohol use, a greater percentage than the deaths caused by HIV/AIDS or tuberculosis. The hazard of alcohol use is greater in men than in women (6.2% vs 1.1%) [1]. It is estimates that 80% of alcoholics were also addicted to nicotine, whereas alcohol dependence increased in 4-10% population of nicotine dependence [2].

However, the biological mechanisms underlying the co-abuse of alcohol and nicotine is complex mechanisms. Nicotine binds specifically to the nicotinic receptors in the brain known as the nicotinic acetyl choline receptors, which have also been recently known to have affinity for alcohol. This theory may underlie co-abuse of these two addictive substances [3].

Relapse is a shared problem in drug dependence. Activation of the mesolimbic dopaminergic system is not only mediated by dopamine but also by other neurotransmitters in the brain. Consequently, relapse may not be only induced by drug to which an individual is addicted but also by other addictive substance due to similarity in neurotransmitters pathway [4].

Opioid system has been known have links to alcohol and nicotine as showed by the use of opiate antagonists in the treatment of alcohol and nicotine dependence [5]. Alcohol as well as nicotine consumptions may cause the release of endogenous opioids in the brain such as beta-endorphin and dynorphin [6, 7].

This may facilitate the rewarding effect of the two addictive substances. Acetylcholinesterase (AChE), the acetylcholine degrading enzyme, could be a marker of cholinergic system as it functions as a receptor and related with acetylcholine release [8]. CPP paradigm used in our present study involves mostly the aspects of learning and memory. Thus mechanisms underlying memory and learning which relate to the cholinergic system that has to be looked at. In this regard, measurement the level of AChE might be warranted.

MATERIALS AND METHODS

Animals

Male Swiss-Webster mice 2-3 months old, weighing about 20-30g obtained from Animal Laboratory School of Pharmacy, Institute of Technology Bandung, Indonesia. The animals had free access to food and water except during experiments. The treatments were carried in accordance with ethics for animals care and use.

Chemicals

All the chemicals used throughout this study were of analytical grade. Alcohol 96% were obtained from Merck, acetylcholine iodide, nicotine hydrogen tartrate, 5, 5-dithiobis-2-nitrobenzoic acids (DTNB) were obtained from Sigma Aldrich, morphine hydrochloride were obtained from Boehringer Ingelheim, sodium di hydrogen phosphate, disodium hydrogenphosphate.

Apparatus

The testing apparatus for the conditioned place preference consisted of three compartments measuring 12.7 cm x 46.5 cm x 12.7 cm (width x length x height) in size. The middle compartment (A) was grey, called neutral compartment. Two conditioning compartments differed in color and floor texture. Compartment B was white with quadrangular sieve (mesh). The other compartment (C) was black with stainless steel floors. Each compartment was separated by two doors.

Behavioral testing

The conditioned place preference method was carried out using biased design, consisting of four phases testing: habituation (5 days), conditioning (5 days), abstinence (5 days) and relapse.

Habituation

Adaptation was aimed to reduce the stress related to the environment includes the weighing room, the testing room, CPP apparatus and the stress due to the injection or the administration of drugs. This phase was conducted in five days.

Preconditioning test

This test was used to determine drug-paired compartment (compartment with lower score preference). One day after habituation each animal was put for 5 minutes in grey compartment and guillotine doors were closed. The doors then opened and animal was allowed to access all compartments for 15 minutes. The time spent by the animal in each compartment was recorded and preference score was calculated using following equation:

$$\text{Preference score} = \frac{\text{Sojourn time in related compartment (second)}}{\text{Total time spent in all compartments (second)}} \times 100\%$$

Conditioning and postconditioning test

In conditioning test, animal was injected with either drug (nicotine 0.5 mg/kg and alcohol 2 g/kg after 30 minutes) or saline than placed in conditioning compartment for 30 minutes. After four hours, animal that injected with the drug in the first session was given saline in the second session and vice versa. On next day, this procedure was reserved, if in first day animal was injected with a drug in the first session, then in second day animal injected with saline. This procedure repeated until five days of trials so that the animal received five drugs and five saline sessions.

Postconditioning test was conducted one day after conditioning test. Preference score was determined using a similar procedure and equation used in preconditioning test.

Abstinence and post abstinence test

This test was carried out in one day after postconditioning test. Procedure of abstinence test was comparable with conditioning test (5 days of trial), but both conditioning compartment were paired with saline. After five days of treatment, post abstinence was conducted with similar procedure used in preconditioning and postconditioning test.

Relapse

Following abstinence test, animals were challenged with different doses of morphine (5, 10, 20 and 40 mg/kg) intraperitoneally. After injected with morphine, score preference each group was calculated.

Acetylcholinesterase activity assay

Ellman's method was used to measure acetyl cholinesterase activity. Brain samples of mice were isolated after conditioning, abstinence and relapse tests.

Brain sample preparation

Following dislocation, the brain was isolated immediately then weighed and washed with saline. If samples were not directly analyzed, they were stored at -70°C temperature [9].

The brain was weighed and homogenized in 0.05M phosphate buffer pH 7.2 using Edmund Bühler homogenizer at a concentration of 20 mg/ml. The aliquot of the brain tissue was incubated at 37°C for 10 minutes. An amount of 400 µL of sample was taken and mixed with 2.6 ml of phosphate buffer, 10 µL acetylcholine chloride and 20 µL dithiobisnitrobenzoic acids (DTNB) [10, 11].

Assay of enzyme activity

The enzyme activity was measured using a spectrophotometer (Beckmen Coulter DU-720) at a wavelength of 412 nm with a kinetic model in which the absorbance was measured for 8 minutes at 1 minute interval.

Calculation of enzyme activity was performed using the formula:

$$R = 5.74 \times 10^{-4} \times \Delta A / Co$$

Where,

R: the rate of substrate hydrolysis (mol/min/g brain tissue)

ΔA: Changes in absorbance per minute

Co: tissue concentration (mg/ml)

Statistical analysis

The result was analyzed statistically using Statistical Package for the Social Sciences (SPSS) software version 18 programmes. Analysis of variance (ANOVA) with post-hoc Tukey LSD was used to analyze the data, and value of p <0.05 and p <0.001 was set for statistical significance.

RESULTS AND DISCUSSION

Effect exposure to morphine on relapse

In this study, animals were conditioned with alcohol (2 g/kg) and nicotine (0.5 mg/kg) intraperitoneally to induce dependent. In post conditioning test, all treatment groups showed increased in preference scores compared to those of preconditioning test. And in relapse test, scores were greater than those in postconditioning test, as showed in fig. 1. This result indicates that the substance given in conditioning and relapse tests (morphine) could induce place preference which has to be in conformity with previous results [12, 13]. Effects of an addictive substance observed through place preference paradigm have been shown to be the outcome of the learning process in animals that were given the stimulus [12].

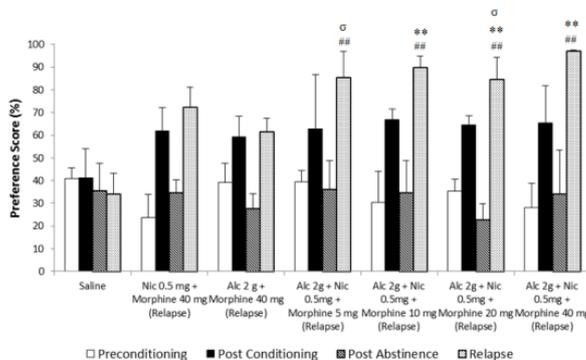


Fig. 1: Preference scores in preconditioning, postconditioning, postabstinence and relapse test. Animals were conditioned (5 days) with alcohol (2g/kg), nicotine (0.5 mg/kg) and combination of both intraperitoneally. Following abstinence period (5 days), they were then challenged with different dose of morphine. ##p<0.001 vs saline, **p<0.001 vs postconditioning score each group, *p<0.05 vs combined group challenged with morphine 40 mg/kg (One way ANNOVA followed by LSD post hoc). Nic = nicotine, Alc = alcohol

As showed in fig. 1, preference score during relapse in animals treated with a combination of alcohol and nicotine was higher compare to animals receiving single dose alcohol or nicotine. This suggests that nicotine could increase reinforcing effect of alcohol and vice versa, as has been shown in previous studies [14-16]. The effect is probably mediated through direct activation of cholinergic neurons by alcohol located in the ventral tegmental area (VTA) of mesolimbic dopaminergic pathway [3, 17]. When drug abstinent animals were challenged with morphine at 10, 20 and 40 mg/kg, the preference score increased significantly compared to postconditioning score (p<0.001). Dependence was mediated by mesolimbic dopaminergic pathway particularly in VTA and the nucleus accumbens (NAc). Activation of this pathway can occur directly through dopaminergic neurons or indirectly through other neurons e. g cholinergic, opioid, glutamate, and serotonin. Alcohol was known to activate dopamine neurons indirectly through alteration of GABA and NMDA receptors [18]. Furthermore, Clapp et al. [19] reported that repeated exposure to addictive drugs in individuals with alcohol dependence caused direct activation of dopamine neurons that were already sensitive due to early exposure. Alcohol has been reported to increase β-endorphin in VTA region [6, 20]. In addition, morphine has been shown increase rewarding effects of alcohol due to activation of mesolimbic dopaminergic pathway indirectly involving GABAergic system located in VTA. Morphine as selective agonist of opioid μ-receptors

was shown to suppress GABAergic neurons, this facilitated dopamine cell firing [21]. Johnson et al. [22] further reported that agonist of μ -receptors caused hyperpolarization of GABAergic neurons. It facilitated inhibition GABA neurons that led excitation of dopamine, further facilitated reinforcing effect. With regard to cholinergic system, it has been reported that nicotine consumption could alter opioid peptides and receptors. Acute nicotine was demonstrated to enhance dynorphin synthesis and release in the striatum accompanied by increased prodynorphin mRNA in caudate and NAc [7]. Subchronic nicotine exposure caused down regulation μ receptors in the hippocampus and striatum significantly [23]. Whereas, chronic administration of nicotine was shown to induce upregulation of μ receptors in the striatum and decrease the level of met-enkephalin in the midbrain. μ -receptors was known to play important role in rewarding effect of morphine [24].

Learning and memory play an essential role in development of dependence in animal subjected to CPP paradigm. When a drug or other rewards gave to animals as unconditioned stimuli, it may result unconditioned responses that would be associated with conditioned stimuli. Conditioned stimuli will result in conditioned responses similar to unconditioned responses which is described in CPP sojourn time [25]. Addiction to substance may establish reward-related learning involving long term and short term memories. Brain substrates which may underlie this reward-related learning is forebrain circuit including VTA, amygdala and prefrontal cortex that receive signal from neurons in midbrain [26, 27].

Alcohol was well known impaired memory, in which cholinergic system is essentially involved. Administration alcohol in adolescent rats at high dose impaired spatial memory but not in lower dose [28]. Hen et al. reported that animals given ethanol and inhibitory GABA transaminase showed effect on acetylcholine level in septohippocampal, part of the brain responsible for memory and learning process. This result indicated that there was involvement of the GABAergic system on memory caused by ethanol [29]. Study in animals given ethanol and naloxone (an opiate antagonist) showed effects on short-term memory, and this might be mediated by endogenous opioid [30]. Midlands et. al reported that administration of μ -opioid receptors antagonist on the CA3 region of hippocampus impaired the acquisition of spatial learning without sensory deficits, suggesting that CA3 μ -opioid receptors play an important role in memory [31]. Further evidence show that there was a link between GABAergic, opioid and cholinergic system in the effect of ethanol. In an experiment using CPP paradigm, animals pre treated with morphine prior to ethanol showed enhanced memory performance and coadministration of ethanol with antagonist of GABAergic, antagonist opioid and cholinergic prevented this effect [4].

Acetylcholine activity assay

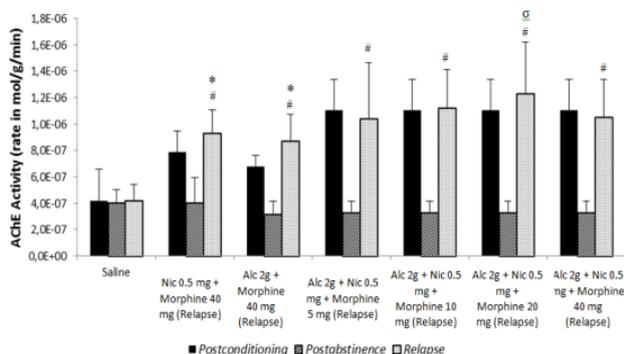


Fig. 2:Acetylcholinesterase activity in the brains of alcohol-depedentanimals. Sample was collected immediately after postconditioning, abstinence and relapse tests. Ellman’s method was used to measure enzyme activity. #p<0.05 vs saline; *p<0.05 vs group treated with 2 g/kg Alc and 40 mg/kg morphine, *p<0.05 vs postconditioning (One way ANNOVA followed by LSD post hoc). Nic = nicotine, Alc = alcohol

Fig. 2 shows that there were significant differences in AChE activity in all groups given the priming dose of morphine compared with saline group (p <0.05). AChE activity in group conditioned with alcohol and nicotine and received priming dose of 20 mg/kg morphine was significantly different compared to group receiving 2g/kg alcohol and challenged with 40 mg/kg morphine. There were no significant differences between the AChE activity in groups conditioned with alcohol and nicotine then given a priming dose of morphine at 5 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg.

Rezayof et al. [13] reported that acetylcholine and AChE antagonists had the effect on the score of preference and locomotor activity as a result of administration of morphine. Furthermore, administration of morphine might influence the release of acetylcholine, the substrate of AChE. Indeed as shown in our present study priming dose of morphine in alcohol dependent animals increased the activity of AChE.

AChE activity in the relapse test was not linear with increasing dose of morphine challenged, where the highest activity was shown in the group given 20 mg/kg morphine priming dose. This finding might be explained by results of studies investigating interaction between administration morphine and acetylcholine level. Taraschenko et al. [32] showed that the acute administration of morphine had a biphasic effect on acetylcholine release. At low doses, morphine increased acetylcholine release, while at higher doses it inhibited the release. Morphine was known to have high affinity to μ receptors and low affinity to κ receptors. Increased acetylcholine release has been shown to be mediated by activation of μ receptors, where as inhibition of acetylcholine release has been related with activation of κ receptors [33].

CONCLUSION

Results of this study show that exposure to morphine increases preference scores during relapse in alcohol dependent animals, and this is accompanied by increased AChE activity. This results further suggests interaction between cholinergic-opioid systems in alcohol dependence.

ACKNOWLEDGEMENT

This research has been partially funded by Innovative Research Scheme of the Institute of Technology Bandung 2013.

CONFLICT OF INTERESTS

Declared None

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