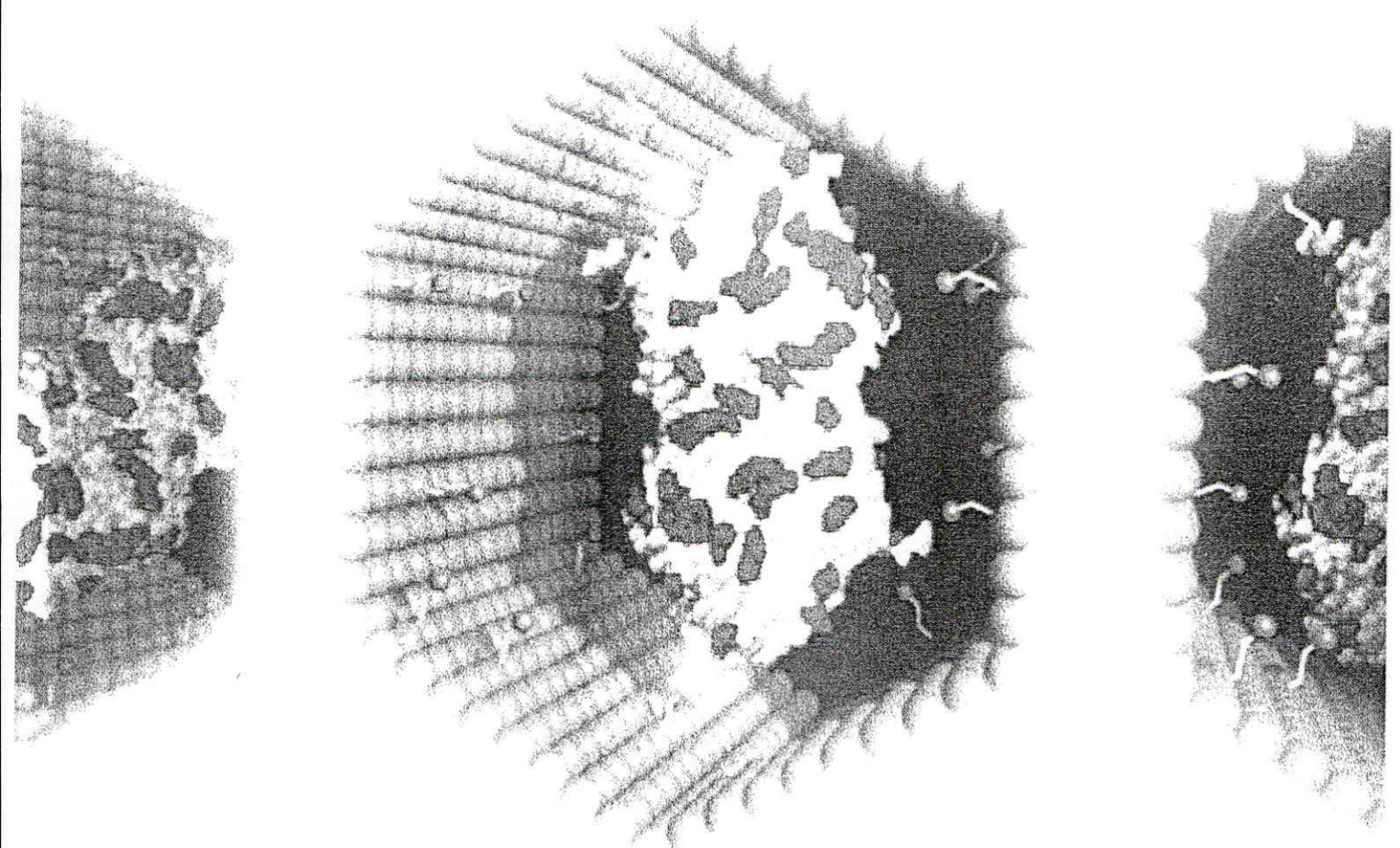


MAJALAH  
FARMASI AIRLANGGA  
(Airlangga Journal of Pharmacy)

ISSN 0852-1050

VOL.7 No.2, OKTOBER 2009



PENERBIT  
FAKULTAS FARMASI UNIVERSITAS AIRLANGGA

## Effect of U-50,488 H on the Development of Drug Craving Behavioral and Protection of Morphological Change in Prefrontal Cortex, Thalamus and Amygdala of the Mice (*Mus musculus*) In the Morphine-Induced Dependence

Aniek Setiya Budiatin, Twoti Dika Parmanasari, Uswatun Khasanah, Junaidi Khotib

Department of Clinical Pharmacy, Faculty of Pharmacy, Airlangga University, Surabaya

Repeated treatment of morphine produces several change in brain and behavior that far outlast their initial neuropharmacological effects. The nature of persistent drug-induced neural plasticity is interested because it is thought to contribute to the development of drug dependency and addiction. The purpose of this study was designed to investigate the effect administration of a selective  $\kappa$ -opioid reseptor agonist (U-50,488H) on rewarding effect associated with morphine in mice and the neuron morphological change on the several brain region (PFC, thalamus and basolateral amygdala). An unbiased conditioned place preference was developed to evaluate the rewarding effect of morphine in this animals. Drug craving model was induced by subcutant injection of morphine at 3 mg/kg BW (based on previous study) once a day for six alternately days. Various doses U-50,488H (0.1 mg/kg; 1.0 mg/kg; 3mg/kg BW) was administered by subcutant injection pretreatment with morphine. The data showed that rewarding effect of morphine was strongly suppressed by pretreatment U-50,488H and significantly attenuated at dose 0.1 mg/kg ( $F_{(4,48)} = 14.024; p < 0.05$ ). The brain tissue was harvested on day 11 and sliced stereotaxically on PTC, thalamus and basolateral amygdala. The slicing was stained with routine histology staining, hematoxyllin eosin for morphological qualitative analysis. The result showed that U-50,488H restore neuron morphology in the several brain region (PFC, thalamus and basolateral amygdala) induced by morphine.

**Keywords :** Conditioned Place Preference; drug craving; morphine; PFC, thalamus and basolateral amygdala; U-50,488H

### INTRODUCTION

There are three major features of addictive behavior need to be explained by any adequate theory drug addiction. The first is drug craving by which the simply mean intensely 'wanting' drugs. The second drug craving often persists or can be reinstated, long after the discontinuation of drug use. An understanding of the propensity to relapse will be critical not only for understanding the process of addiction, but in developing effective therapies. A third feature of drug addiction that requires explanation is that, as drugs come to be 'wanted' more-and-more, they often come to be 'liked' less-and-less. That is, as craving for drugs increases the pleasure derived from drugs often decreases. Why is this? What is the relationship between 'wanting' drugs and 'liking' drugs and does this relationship change during addiction? (Robinson1993).

The central feature of drug addiction is compulsive drug use—loss of control over apparently voluntary acts of drug seeking and drug taking (Glare, 1991). Addiction is a chronic disorder, since even after treatment and extended periods of drug abstinence, the risk of relapse to active drug use remains high. In this review, we consider some molecular mechanisms and neural circuits that may be involved in persistent, compulsive drug abuse. We do not attempt to provide a comprehensive account of the numerous effects of addictive drugs on the brain Another approach, which has been explored for potential treatments of stimulant abuse, is the use of  $\kappa$ -opioid agonists. The  $\kappa$ -receptor is known to be involved, via indirect effects, in synaptic dopamine levels (Figure 1). This review covers several classes of  $\kappa$ -opioid ligands that have been explored for this purpose (Prisinzano, 2005). Repeated treatment of morphine produces several

changes in brain and behavior that far outlast their initial neuropharmacological effects. The nature of persistent drug-induced neural plasticity is interested because it is thought to contribute to the development of drug dependency and addiction. The purpose of this study was designed to investigate the effect of administration of a selective  $\kappa$ -opioid reseptor agonist (U-50,488H) on rewarding effect associated with morphine in mice and the neuron morphological change of the several brain region (PFC; thalamus and basolateral amygdala). An unbiased conditioned place preference was developed to evaluate the rewarding effect of morphine in these animals

### Objective

The purpose of this study was to investigate the effect of administration of a selective  $\kappa$ -opioid receptor agonist (1 S-trans)-3,4-dichloro-N-Methyl-N-[2-(1-pyrrolidiny)-cyclohexyl]benzeneacetamide hydrochloride [(-\_U-50,488H)] on rewarding effect and structural changes of dopaminergic neurons associated with chronic morphine treatment in mice. Male balb/C mice (weighing 25-30 g) was adapted into three consecutive phases of unbiased CPP procedure. Male mice; genus *Mus*, subfamili *Murinae*, famili *Muridae*, ordo *Rodentia*, spesies *Mus musculus*

Presented in The 2<sup>nd</sup> Collaborative Conference UNAIR-USM, February, 10<sup>th</sup> - 11<sup>th</sup> 2009, Airlangga University, Surabaya, Indonesia

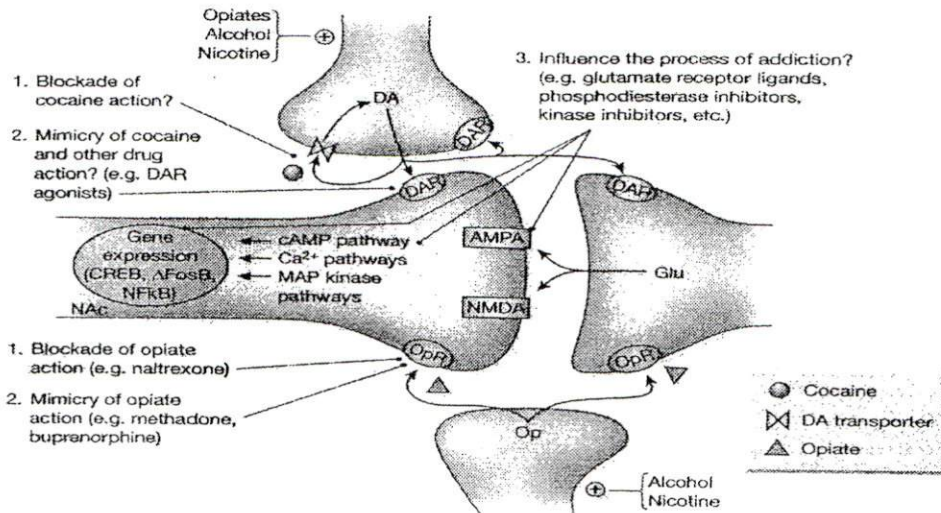


Figure 1. Treatment strategies for opioid dependence

**MATERIALS AND METHODS**

**Materials**

CPP box, microtome, Rotarod (UGO Basile, Italia), microscope, microbalance, stopwatch, morphine HCl (PT. Kimia Farma, pharmaceutical grade), lithium carbonate, U-50,488H (Sigma, analytical grade), haematoxyline eosin, normal saline steril, ether, aqua pro injection, paraffin, neutral buffered formalin, 1%-acidic alcohol.

**Methods**

Male Balb/C mice (weighing 25-30 g) was adapted into three consecutive phases of unbiased CPP procedure. On pre-conditioning phase, mice were allowed to freely explore all of the compartments for a 900-sec period to see

the preference of each individual mice (Figure 2). On conditioning phase, these animals alternately received a single injection s.c of saline, morphine 3.0 mg/kg, or pretreatment of various dose of U-50,488H (0.1mg/kg; 1.0 mg/kg; 3.0 mg/kg) and were confined to the compartment for 60-min. On post-conditioning, test day was evaluated to determine compartment preferences (Bardo, M.T, 2000). In this study, the sedative effect of administration morphine and pretreatment U-50,488H was measured using a RotaRod apparatus for a 60-sec period. To evaluate the effect of administration morphine and pretreatment U-50,488H at the projection of dopaminergic neuron especially at basolateral amygdala and thalamus, the brains were sliced 5 µm tight then were stained by hematoxyline eosin and identified by light microscope.

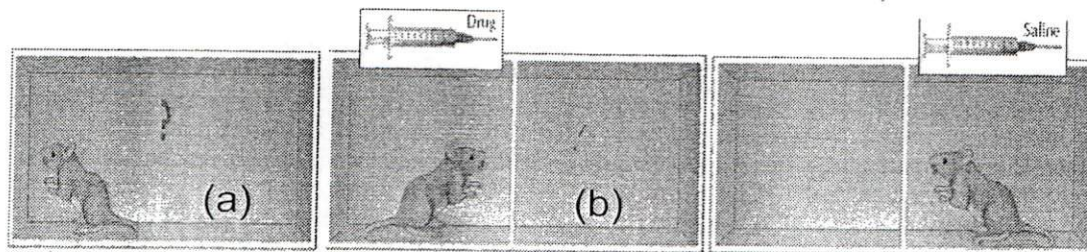


Figure 2 Pre-conditioning (a) and post - conditioning (b)

**RESULTS AND DISCUSSION**

The data showed that the rewarding effect of morphine was suppressed by pretreatment of U-50,488H (0.1mg/kg; 1.0 mg/kg; 3.0 mg/kg, s.c) with morphine and was significantly attenuated at dose 0.1 mg/kg respectively ( $F(4, 48) = 14.024; p < 0.05$ ) (Table 1). Both U-50,488H and morphine had no significant difference on sedative effect in mice (Table 2). Administration of chronic

morphine resulted structural changes at the region include reduction of cells size, quantity of the cells, and morphological changes. In line with its ability to suppress the rewarding effect of morphine, the change in those brain regions was prevented by the concomitant pretreatment of selective κ-opioid receptor agonist, U-50,488H (Figure 3 and figure 4).

Table 1. Score CPP (second) for morphin group vs U-50,488H

Group	Scor CPP (Mean ± SE)
Negative Control	45.27 ± 20.48
Morphin 3 .00 mg/kg BB	167.25 ± 31.68
U50,488H 0.1 0 mg/kg BB	-5.18 ± 27.64
U50,488H 1 .00 mg/kg BB	-93.25 ± 23.63
U50,488H 3 .00 mg/kg BB	-64.91 ± 26.94

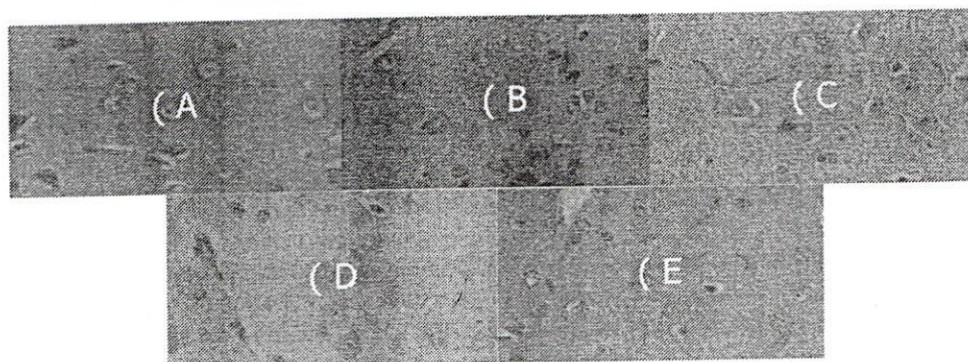


Figure 3. Basolateral amygdala (BLA) with hematoxyllin eosin staining, (a) Control; (b) morphine, (c) U-50,488H 0.1 mg/kg, (d) U-50,488H 1 mg/kg, (e) U-50,488H 3 mg/kg ; 400x normal size.

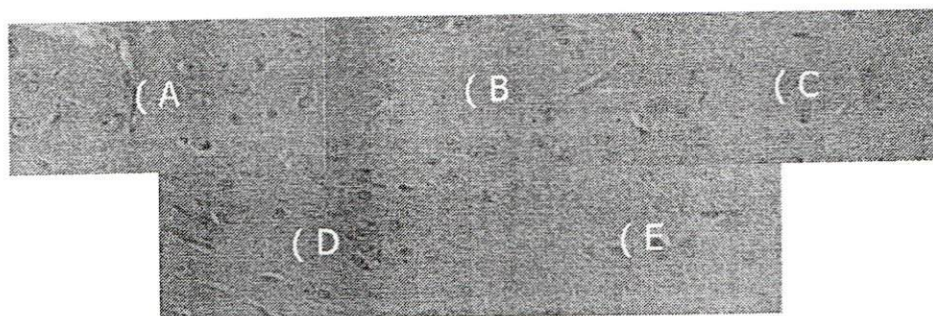


Figure 4. Talamus with hematoxyllin eosin staining, (a) Control; (b) morphine, (c) U-50,488H 0.1 mg/kg, (d) U-50,488H 1 mg/kg, (e) U-50,488H 3 mg/kg ; 400x normal size

Table 2. The influence of U-50,488H treatment to the mice's coordination function. There were no significant difference with ANOVA one way direction analysis ;  $F(4,41) = 0,891; (p < 0.05)$ .

Group	Resistance time (seconds) (Mean ± SE)
Morphine 3.00 mg/kg	60.00 ± 0.00
U-50,488H 0.10 mg/kg	60.00 ± 0.00
U-50,488H 1.00 mg/kg	60.00 ± 0.00
- U 50,488H 3.00 mg/kg	58.56 ± 4.33

The opioid  $\mu$  receptor activation provided the increased locomotor, euphoria effect and drug craving, which the opioid  $\kappa$  receptor showed the dysphoria, psychotomimetic effect, sedative and drug refused (Narita *et al*, 2003; Khotib *et al*). The result was showed the effect of administration of a selective  $\kappa$ -opioid reseptor agonist (U-50,488H) on rewarding effect associated with morphine in mice and the neuron morphological change of the several brain region (PFC, thalamus and basolateral amygdala). Administration of chronic morphine resulted structural changes at the region include reduction of cells size, quantity of the cells, and morphological changes ( Figure

3b and figure 4b) and selective  $\kappa$ -opioid reseptor agonist (U-50,488H) inhibited the morphology changes and cell size ( Figure 3 c;3d and figure 4c ;4d)

### Conclusion

The present study clearly demonstrate that  $\kappa$ -opioid reseptor agonist U-50,488H might be a value in the treatment and prevention of relapse to drug craving behavior associated with morphine, and also prevent the remodeling of morphological neurons that associated with rewarding process .

## REFERENCES

- Bardo, M.T and Bevins, R.A., 2000. Conditioned place preference: what does it add to our preclinical understanding of drug reward ?. *Psychopharmacology*, Vol.153 No.1, p.31-43
- Glare, P.A and Walsh, T.D., 1991. Clinical Pharmacokinetics of Morphine. *The Drug Monit*, Vol.13 No.1, p.1-23
- Glare, P.A and Walsh, T.D., 1991. Clinical Pharmacokinetics of Morphine. *The Drug Monit*, Vol.13 No.1, p.1-23
- Khotib, J., Zulkarnain, S.B., Yulistiani., Syamsiah, S dan Suzuki, T., 2005. Peran Agonis Reseptor Opioid Kappa (-) U-50,488H Dalam Penanganan Gejala Putus Obat Morfin dan Metamfetamin. *Abstrak Presentasi Oral : Bidang Biomedik*
- Narita, M., Khotib, J., Suzuki, M., Ozaki, S., Yajima, Y and Suzuki, T., 2003. Heterologous  $\mu$ -opioid receptor adaptation by repeated stimulation of  $\kappa$ -opioid receptor: up-regulation of G-protein activation and antinociception. *Journal of Neurochemistry*, Vol. 85, p.1171-1179
- Prisinzano, T.E., Tidgewell, K and Harding, W.H., 2005.  $\kappa$ -Opioids as Potential Treatment for Stimulant Dependence, *The AAPS Journal*, vol.7 No.3, p.92-99
- Robinson Terry E and Berridge Kent C., 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction, *Brain Res Rev*, 18 (1993) 247-291.