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Full Length Research Paper

The effect of propranolol and metoprolol on memory performance during morphine withdwal in mice

Azadeh Mesripour*, Mahmoud Rafieian-kopaei, Roya Ansari

Medical Plants Research Centre, Shahrekord University of Medical Sciences, Shahrekord, IRAN

*Corresponding author email: a_mesripour@yahoo.com or mesripour@skums.ac.ir; Tel: +98 381 3335652; Fax: +98 381 3330709.

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Beta blockers were used to see if they could prevent the destructive effects of GCs on memory acts. Cognition was evaluated using the object recognition task, the difference in the exploration time between a familial (F) object and a novel (N) object was taken as an index of recognition (RI). Male mice were made dependent by increasing doses of morphine twice daily for three days; RI was assessed after naloxone precipitated withdrawal on the third day. Propranolol and metoprolol were used before the test trial in morphine withdrawn animals. These drugs were also administered as a 3 day protocol. Memory performance was impaired in morphine dependent animals, however neither propranolol nor metoprolol single dose, or three days protocol showed no improvement in memory performance. Beta blockers effects on other aspects of recognition for instance sedation and anxiety may augment recognition loss.

Keywords: morphine; withdrawal; memory; β-blocker; object recognition task

INTRODUCTION

Several lines of evidence indicate that opiates modify learning and memory processes (Peart, Gross *et al.,* 2005; Bodnar and Klein 2005). It is also well known that learning and memory are critically involved in the morphine dependence and relapse (Robbins and Everitt 1999). Morphine impaired memory acquisition in the radial maze (Spain and Newsom 1991) and water maze (Li, *et al.,* 2001). Using the object recognition task it was also observed that recognition memory was impaired in dependent mice following morphine withdrawal (Mesripour *et al.,* 2008).

Morphine withdrawal is associated with activation of the hypothalamic pituitary adrenocortical axis (Morley, 1981). Glucocorticoid measurements in serum and brain samples after morphine withdrawal also showed considerable increase in glucocorticoid concentration compared with controls (Rabbani *et al.,* 2009).

Findings demonstrate that after memories are consolidated, the efficacy or accuracy of the information retrieved is vulnerable to glucocorticoid influences at the time of recall (Roozendaal, 2003). Furthermore noradrenergic activity of the basolateral amygdala is critical for enabling glucocorticoid effects on memory consolidation and retrieval (Roozendaal *et al.,* 2003). Due to a rich and widespread projection of adrenergic axons, it is thought that norepinephrine (NE) plays a modulatory role in some aspects of brain function (Jurgens *et al.,* 2005). Evidently NE plays an important role in regulating memory storage through a beta adrenergic receptor-mediated mechanism (Ferry *et al.,* 1999).

The effect of beta receptors on memory performance after morphine withdrawal, and the elevated level of corticosterone concentration are not clear yet. Therefore in the present study the effects of propranolol ($\beta_{1,\ 2}$ antagonist) and metoprolol (β_1 selective antagonist) were evaluated on memory performance in morphine dependent animals after withdrawal. Since beta blockers have proved to be useful in controlling stress disorders, therefore they may also be useful to decrease corticosterone induced memory impairment following morphine withdrawal in dependant animals. If such these

results could open new windows toward improving drug therapy following morphine withdrawal in patients.

MATERIALS AND METHODS

Animals. Male Balb/C mice weighing 25-30 g were housed in cages of six at 21± 2 ^{oC} in a 12 h light-dark cycle with the lights on at day time 6 am-6 pm. Tap water and standard food pellets were available ad libitum. Tests were performed only after the mice had acclimated to the above environment for at least 2 days. In order to minimize circadian rhythm influence, all experiments were conducted between 08:00 and 13:00 h, in a special noise-free room with controlled illumination. Minimum of six mice were used for each treatment group. All procedures were approved by the Ethical Committee of the University of Medical Sciences, and conducted in accordance with the 'Principles of Laboratory Animal Care' (National Institutes of Health publication no. 86-23, revised 1985).

In order to evaluate the effect of morphine withdrawal induced stress on the adrenal, animals were euthanized using di-ethyl ether for less than 3 min and rapidly after laparotomy their adrenals were collected and placed on water absorbent paper to absorb the extra blood and they were weighed rapidly and measured as mg/g body weight.

Object recognition task. The object recognition task was performed as described previously (Mesripour *et al.,* 2008). Briefly, square wooden open-field (35×35×40 cm) with the inside painted in dark black and a white floor was used. It was placed in a dark room with a uniform dim light toward the apparatus.

At the first day, animals were submitted to a habituation session in the open field and allowed to freely explore the arena in the presence of two objects for at least 15 min. On experimental day, animals were submitted to two trials spaced by an intertrial interval (20 min). During the first trial (acquisition trial, T1), animals were placed in the arena containing two identical objects for an amount of time necessary to explore the objects for 20 s. Any mouse not exploring the objects for 20 s within the 12-min period was excluded from experiments. Exploration is defined as the animal directing the nose within 2 cm of the object while looking at, sniffing, or touching it. For the second trial (test trial, T2), one of the objects presented in the first trial was replaced by new object, animals were placed back in the arena for 5 min and total time spent in exploration of each object was determined. Animals behavior were recorded by using a web camera mounted above the experimental apparatus, records were analyzed later.

Drug treatments. Mice were made dependent by increasing doses of morphine sulfate (Temade co., Tehran, Iran), twice daily with 12 hour intervals; 30 and 45 mg/kg at the first day, 60 and 90 mg/kg at the second day, and 90 mg/kg the last day (Mesripour *et al.,* 2008) . Time interval elapsed between the last morphine dose and naloxone injection was 3 h, naloxone (Tolid Daru Co., Tehran, Iran) was injected after T1 (in the morphine+ naloxone and naloxone alone groups). Twenty minutes after naloxone injection mice were tested in the memory apparatus (T2). Propranolol and metoprolol (Darou-pakhsh, Tehran, Iran) (2 mg/kg) were injected 40 min before T2 and control animals received saline.

The effect of 3 days propranolol and metoprolol (2 mg/kg) were also determined on memory performance after morphine withdrawal. In this regard animals received a single dose of propranolol, metoprolol or saline each day 2 hour after morphine injection. The timing of injections at the last day was as mentioned earlier. The effect of propranolol and metoprolol alone on memory performance was also determined.

Data processing and statistical analysis. The following parameters were measured: time required to achieve 20 s of object exploration on T1 (duration of T1), time spent in active exploration of the familiar (F) or novel (N) object on T2. Recognition memory was evaluated using a recognition index (RI) calculated for each animal using the formula: (N−F/N+F)×100 corresponding to the difference between the time exploring the novel and the familiar object, corrected for total time exploring both objects (Bertaina-Anglade *et al.,* 2006). Positive values indicate a good discrimination performance, while negative values or those around zero indicate very poor discrimination capacity.

Results were expressed as group mean ± SEM. All results were analyzed by a one-way analysis of variance (ANOVA), followed by Duncan's multiple comparison tests, P values less than 0.05 were considered significant. The software used for data analyzing and making graphs was the GraphPad Prizm 5.

RESULTS

The effect of treatments on the learning procedure. At the first trial or the learning period the time required for 20 s object exploration was determined. Control animals recognize the objects rapidly (T1= 2.6 min ± 0.42).

Propranolol and metoprolol were used in normal animals and the results were compared with vehicle (Table 1). At the first trial although propranolol and metoprolol increased the recognition time (T1) to 3.3min ± 0.3 and 3.2min ± 0.7 respectively but it was not

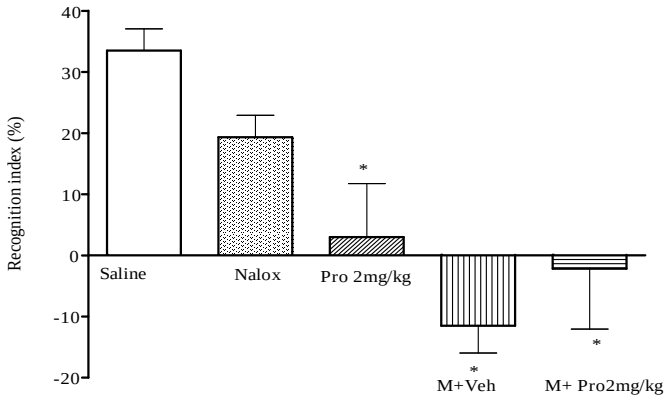

Figure 1. The effect of propranolol single dose (sc, 40 min before T2) alone together with its effect on memory performance after naloxone (0.1mg/kg, ip) precipitated morphine withdrawal on day 3. This figure also shows the effect of naloxone alone on RI. Memory is expressed as recognition index RI= (N−F/N+F) ×100, in the novel object recognition task. Vehicle treated animals received saline. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Duncan's multiple comparison tests. *P< 0.05 compared with control values, that received only saline (blank bar). Number of animals in each group was 6.

considerably differed compared with control. Morphine dependent animals also required more time to recognize the objects but this was not significant (T1= 5min ± 1.6).

The effect of beta antagonists alone on memory performance. Propranolol or metoprolol (2 mg/kg) were injected 40 min before T2. These drugs affected memory performance at the second trial. As can be depicted in Figure 1, RI- significantly reduced to 3.3% ± 0.33 (P<0.05) in animals treated with 2 mg/kg propranolol, that is animals did not distinguish between the new and the familial object. Treating animals with 2 mg/kg metoprolol also caused a drop in RI (-2.9% ± 8.4) compared with saline values accounting for RI= 34% ± 3.6, P<0.05 (Fig. 2). Therefore memory performance was influenced by the above drugs in the NOR task. As it is presented in figures 3 and 4, three days treatment with propranolol or metoprolol (2 mg/kg) also impaired memory performance (RI=-5.5% ± 4.3, RI=-8.5% ± 3.1 respectively, P<0.05).

The effect of beta antagonists' single dose on RI during withdrawal. Figure 1 illustrates RI scores following the concurrent treatments. Memory index in animals that received 2 mg/kg propranolol was -2.2% ± 9.9 that alike vehicle values (RI= -12% ± 4.5) indicate memory damage, these values significantly differed with the control values (RI= 34% ± 3.6, P< 0.05).

As can be depicted in Figure 2, administration of metoprolol 2 mg/kg following withdrawal did not improve memory performance (RI= 3.5% ± 11) same was observed in the vehicle group (RI= -2.4% ± 5, P< 0.05). Therefore propranolol or metoprolol could not ameliorated memory performance after naloxone withdrawal in morphine dependent animals.

The effect of 3 days injection of beta antagonists on RI during withdrawal. Figure 3 demonstrates the effect of daily propranolol treatment on the RI during morphine withdrawal. The simultaneous daily administration of propranolol 2 mg/kg with morphine did not improve RI =1.5% ± 5.

The effect of daily metoprolol on the RI is shown in figure 4. The concurrent daily administration of metoprolol 2 mg/kg with morphine also could not have any useful impact on the corresponding values (RI=5.5% ± 7). memory performance was not improved according to the low values of RI.

Adrenal gland weights. After animals were euthanized adrenal glands were excised and measured as mg/g body weight. As presented in table 1 after naloxone precipitated morphine withdrawal there was no difference between adrenal weights in either of the groups.

DISCUSSION

Based on our experiments memory performance was impaired in morphine dependent animals compared to control values. Neither Propranolol nor metoprolol single

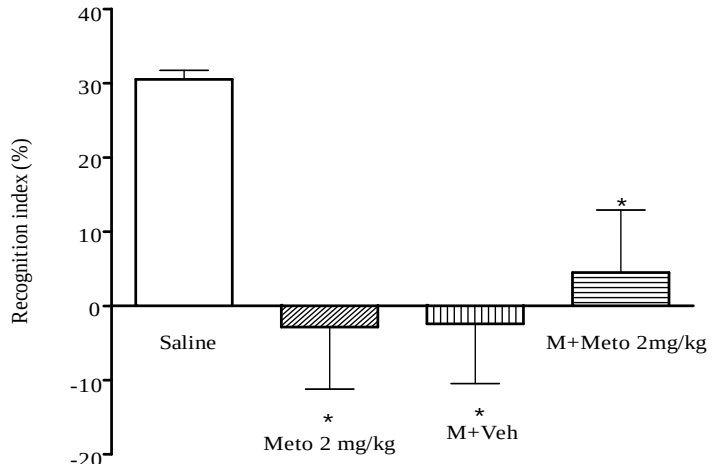

Figure 2. The effect of metoprolol single dose (sc, 40 min before T2) alone together with its effect on memory performance after naloxone (0.1mg/kg, ip) precipitated morphine withdrawal on day 3. Memory is expressed as recognition index RI= (N−F/N+F) ×100, in the novel object recognition task. Vehicle treated animals received saline. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Duncan's multiple comparison tests. *P< 0.05 compared with control values, that received only saline (blank bar). Number of animals in each group was 6.

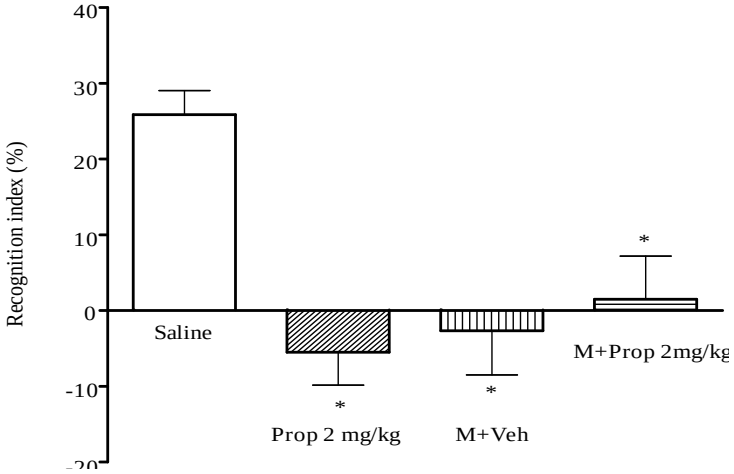

Figure 3. The effect of 3 days propranolol injections alone together with its effect on memory performance after naloxone (0.1mg/kg, ip) precipitated morphine withdrawal on day 3. Memory is expressed as recognition index RI= (N−F/N+F) ×100, in the novel object recognition task. Vehicle treated animals received saline. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Duncan's multiple comparison tests. *P< 0.05 compared with control values, that received only saline (blank bar). Number of animals in each group was 6.

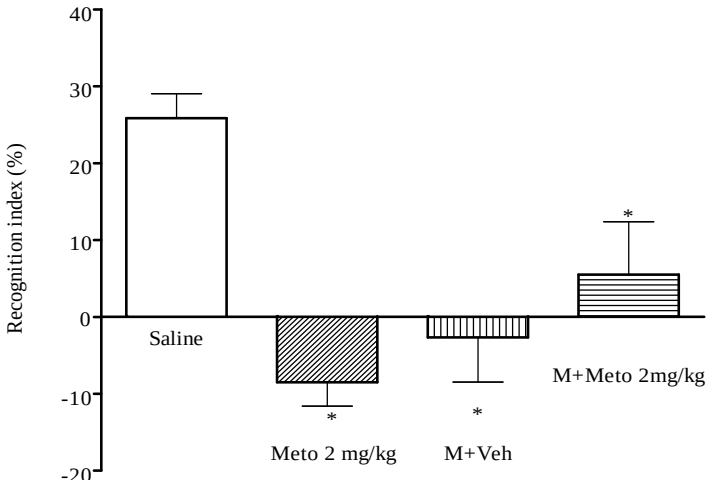

Figure 4. The effect of 3 days metoprolol injections alone together with its effect on memory performance after naloxone (0.1mg/kg, ip) precipitated morphine withdrawal on day 3. Memory is expressed as recognition index RI= (N−F/N+F) ×100, in the novel object recognition task. Vehicle treated animals received saline. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Duncan's multiple comparison tests. *P< 0.05 compared with control values, that received only saline (blank bar). Number of animals in each group was 6.

Table 1. Effect of beta blockers on adrenal weight and on learning performance

	Saline	**propranolol**	**metoprolol**	**Morphine +propranolol**	**Morphine +metoprolol**	**Morphine +vehicle**
Adrenal weight mg/g	0.1± 0.0	0.15 ±0.0	0.11 ±0.0	0.17 ±0.01	0.11± 0.0	0.12 ±0.0
T1 (min)	2.6±0.4	3.3±0.3	3.2±0.7	5.8±1.4	3.8±0.6	5±1.6

The effect of naloxone (0.1 mg/kg, ip) precipitated morphine withdrawal on adrenal weight in morphine dependent animals. The amount of time necessary to explore the objects for 20 s was measured as the T1 value. Vehicle treated animals received saline. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Duncan's multiple comparison tests. There were no difference concerning adrenal weight or learning performance among groups. Number of animals in each group was 6.

dose, or daily dose showed no improvement in memory performance, compared to vehicle group in morphine dependent animals.

In order to evaluate the validity of this memory paradigm scopolamine (0.5 mg/kg) was used. Learning did not differ from control animals but memory performance was impaired. These experiments proved that in our experimental condition this memory apparatus could show the memory performance in mice. As shown previously, naloxone 0.1 mg/kg did not impair memory by its own (figure 1) and the effect of naloxone observed in morphine dependent animals is due to induction of withdrawal (Mesripour *et al.,* 2008). Since zero or values of RI under zero indicate memory impairments, thus in these experiments memory was impaired after withdrawal on the last day (day 3).

This experiment showed that propranolol and metoprolol impaired memory performance. Norepinephrine or the β-adrenoceptor agonist clenbuterol infused into the BLA immediately post training enhanced the memory of many emotionally arousing training tasks, including inhibitory avoidance (Ferry *et al.,* 1999), contextual fear conditioning (Huff *et al.,* 2005) and water-maze spatial training (Hatfield and McGaugh 1999). In contrast, post training intra-BLA infusions of β-adrenoceptor antagonists impaired the consolidation of memory for emotionally arousing training experiences (Miranda *et al.,* 2003). Therefore noradrenergic activities

in brain regions remain important also for memory retrieval.

Memory impairment observed following morphine withdrawal in mice, did not recover after using a single dose of propranolol or metoprolol at the last day of the experiments. The same results were observed when beta blockers were used on three consecutive days in addition to morphine consumption. Among the selective beta blockers metoprolol proves to have higher lipid solubility, thus higher volume of distribution and CNS penetration (Hoffman, 2004). The BLA is a critical site for enabling glucocorticoid effects on memory retrieval (Roozendaal, 2003). Findings indicate that noradrenergic activity of the BLA is critical for enabling glucocorticoid effects on memory consolidation and retrieval (Roozendaal, 2003). Results indicate that beta1- and beta2- adrenoceptors in the BLA have differential subcellular localizations and both are required for the consolidation of auditory fear memory (Qu *et al.,* 2008). Adrenergic signaling is important for the retrieval of intermediate-term contextual and spatial memories. The role of norepinephrine in retrieval requires signaling through beta1-adrenergic receptors in the hippocampus (Gliebus and Lippa, 2007). Therefore it seems that beta receptor activation is not only useful for memory consolidation (Wouda *et al.,* 2010) but in case of morphine withdrawal it may also be useful for object recognition and memory retrieval.

Measuring the adrenal weight in comparison to the animals' body weight did not show any differences among them. Thus although there is a stress load following morphine withdrawal but according to our experiments (Rabbani *et al.,* 2009) and the previous studies, increased corticosterone after morphine withdrawal is because of adrenal sensitivity to ACTH rather than adrenal enlargement.

While in this experiment memory was not improved by beta blockers after morphine withdrawal but this could open new views toward treatment of morphine addicts. Therefore disrupting drug-associated memories could be an important new strategy for treating addictive behaviors. Morphine withdrawal can cause stress symptoms and on the bases of this study and previous studies (Mesripour *et al.,* 2007; Mesripour *et al.,* 2008) it seems that direct effect of increased corticosterone on memory impairment is much more important.

We highlight, although elevated corticosterone level after withdrawal influence the β-adrenoceptor in the BLA, beta blockers effects on other aspects of recognition for instance sedation and anxiety may hinder recognition following morphine withdrawal. In addition although corticosterone and noradrenergic systems are synergistic in improving memory consolidation this effect does not happen in memory retrieval in the novel object recognition task.

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