

The effects of spironolactone on morphine withdrawal induced memory loss by the object recognition task method in mice

A. Mesripour¹, V. Hajhashemi² and M. Rabbani^{2,*}

¹Isfahan Pharmaceutical Sciences Research Center, Isfahan University of Medical Sciences, School of Pharmacy and Pharmaceutical Sciences, Isfahan, I.R.Iran.

²Department of Pharmacology, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R.Iran.

Abstract

Previous reports showed that elevated levels of glucocorticoids following morphine withdrawal play an important role in memory impairment. In addition, glucocorticoid receptor (GR) inhibitors improved memory performance in morphine withdrawal mice. Since mineralocorticoid receptor (MR) and GRs complement each other, the aim of the current study was to evaluate the effects of spironolactone on memory performance after withdrawal in morphine dependent mice. To assess memory performance, the object recognition task was used. Novel object recognition task was carried out in a square wooden open-field apparatus using objects. The test was comprised of three sections: habituation for 15 min, first trial for 12 min and test trial for 5 min. In this learning paradigm, the difference in exploration between a previously seen object and a novel object is taken as an index of memory performance (recognition index, RI). Male mice were made dependent by increasing doses of morphine (30-90 mg/kg) subcutaneously twice daily for three days. Withdrawal was elicited either by injection of naloxone (0.1 mg/kg) 3 h after last morphine injection or spontaneously 4 h after the last dose of morphine on the third day. Spironolactone (50, 100 mg/kg) was used subcutaneously before the first trial and the effects were compared with control values. After naloxone precipitated withdrawal spironolactone at 50 and 100 mg/kg improved RI to $10.8\% \pm 6.0$ and $24.0\% \pm 6.1$ which were significantly different from vehicle ($RI = -24.1\% \pm 6.6$, $P < 0.05$). Following spontaneous withdrawal, spironolactone at 50 mg/kg improved RI to $18.0\% \pm 13.0$ that differed significantly from vehicle ($RI = -20.8\% \pm 11.4$, $P < 0.01$). Results of these experiments show that MRs may play an important role in the recognition memory impairment following morphine withdrawal in mice.

Keywords: Morphine withdrawal; Memory impairment; Corticosterone; Mineralocorticoid receptor; Spironolactone

INTRODUCTION

Morphine and most other opioid agonists affect a wide range of physiological system (1,2). Modulation of learning and memory processes by morphine and other opioidergic agents has been demonstrated in many studies (3-5). Previous reports have shown that acute administration of opioids impairs learning and memory processing (6-8), which can

be attenuated by naloxone (4,9). On the other hand, chronic exposure to opiates can result in an impaired performance on memory task in rats (10,11).

Chronic misuse of opiates will lead to long-lasting impairments in brain function (12). Studies have indicated that many brain areas, particularly the frontal and temporal lobes, are hypofunctional during both prolonged abstinence and acute withdrawal (13,14). This may relate to the

*Corresponding author: M Rabbani
Tel. 0098 311 7922646 Fax. 0098 311 6680011
Email: rabbani@pharm.mui.ac.ir

cognitive deficits found, after the physical symptoms of withdrawal have dissipated (15).

Emotionally arousing experiences activate the hypothalamic pituitary-adrenocortical axis (HPA), resulting in elevated glucocorticoid (GC) levels (i.e., corticosterone and cortisol).

Morphine withdrawal is associated with activation of the HPA (16). A study has shown that animals undergoing acute (12 h) morphine withdrawal displayed a potentiated and prolonged corticosterone response to restraint (17). A longstanding history of studies has demonstrated the ability of GCs to influence memory (18). It has long been recognized that prolonged exposure to stress, impairs memory function in both animal and human subjects. It is now also known that GCs have acute influences on memory. Evidence from different experiments have shown enhancing as well as impairing effects following acute stress or GC treatment (18). The consequences of GC activation on memory depend largely on the different memory phases investigated, i.e., memory consolidation is enhanced by acute stress, while retrieval is impaired (19).

The hippocampus serves a pivotal role in memory formation (20). It has been suggested that corticosteroid modulation of hippocampal activity and plasticity may underlie some aspects of acute and possibly chronic effects of stress (21). Two types of corticosteroid receptors have been described in the brain: mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). The high affinity MRs are most densely localized in hippocampal and septal neurons. GRs are ubiquitously distributed in the brain, including neurons in the hippocampus, hypothalamus, glial cells, and pituitary cells (22). Both MRs and GRs complement each other and put them in a position to modulate the HPA

responsively under stressful conditions (21).

Basal corticosteroid levels are associated with the effective induction of longterm potentiation (LTP) in the hippocampus (23). In contrast, elevated levels of corticosteroid hormones (presumably occupying both GRs and MRs) or exposure to stress have been reported to impair LTP (23). Furthermore, it has been suggested that the MRs and GRs function in a binary manner at the cellular level (24). GR activation seems to involve the suppression or normalization of network activity (24). Conversely, MR activity is considered to maintain the excitability and stability of networks. It has been shown that both acute and continuous spironolactone blockade of MR impair retention of spatial memory, supporting a positive effect of MR activation on cognition (25).

Using the object recognition task, following morphine withdrawal we observed memory impairment in dependent animals (26). Determination of corticosterone concentration in animals showed that morphine withdrawal increased corticosterone level in blood. However, memory was reversed to that of control levels following the administration of metyrapone (corticosterone synthesis inhibitor) and mifepriston (GR antagonist) (26).

The correlation between MRs and the opioid system on memory is not clear yet. Therefore, it would be of interest to determine the effect of spironolactone (MR antagonist) on memory performance after morphine withdrawal. Memory is assessed by the novel object recognition task that originally developed by Ennaceur and Delacour, and it is based on the natural tendency of rodents to explore a novel object more than a familiar one (27).

MATERIALS AND METHODS

Animals

Male NMRI mice (Pasteur institute, Tehran, Iran) weighing 25-30 g were housed in cages of six at 21 ± 2 °C in a 12 h light-dark cycle. 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 Isfahan University of Medical Sciences, and conducted in accordance with the internationally accepted principles for laboratory animal use and care.

Object recognition task

The object recognition task was performed as described by Bertaina-Anglade et al. (28). Briefly, the apparatus was made of a square wooden open-field ($35 \times 35 \times 40$ cm) with the inside painted in dark black and a white floor. The open field was placed in a dark room illuminated only by a halogen lamp oriented towards the ceiling and giving a uniform dim light in the apparatus.

The open field and the objects were cleaned between each trial using water to avoid odor trails. The objects were legos that were different in shapes and colors. Animals were placed in the experimental room at least 30 min before testing.

The day before the test, each animal was 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 a 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.

Drugs

Drugs used in this study were morphine sulfate (Temade, Tehran, Iran), naloxone HCl (Tolid Daru, Tehran, Iran), spironolactone (Sigma, USA). All drugs were dissolved in 0.9% saline just before the experiment, except for spironolactone that was suspended in 0.9% saline by tween 80 (1% v/v). Control animals received either saline or vehicle. Naloxone was injected i.p., and other drugs were injected s.c. The doses were adjusted such that each animal received a volume of 10 ml/kg.

Drug treatments

Mice were made dependent by increasing doses of morphine twice daily with 12 h intervals; 30 and 45 mg/kg on the first day, 60 and 90 mg/kg on the second day, and 90 mg/kg on the last day (29,30). As a control, one group of mice was treated with saline twice daily for three days. Withdrawal was performed in two ways. In one series of experiment, withdrawal was elicited by injection of naloxone (0.1 mg/kg, i.p.) 3 h after last morphine injection. Animals got their first trial before induction of withdrawal. In

another series of experiment, memory performance was determined by spontaneous morphine withdrawal (that is 4 h after the last dose of morphine) in dependent animals. Spironolactone (50, 100 mg/kg) was injected 40 min before T2 and control animals received vehicle (1% (v/v) tween 80 in saline).

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: $(N-F/N+F) \times 100$ corresponding to the difference between the time exploring the novel and the familiar object, corrected for total time exploring both objects (28). Results were expressed as the mean \pm S.E.M.

All results were analyzed by a one-way analysis of variance (ANOVA), followed by Duncan's multiple comparison test, and P values less than 0.05 were considered significant.

RESULTS

Naloxone withdrawal

At the first trial morphine dependent animals receiving vehicle or spironolactone 50 and 100 mg/kg spent 6.1 ± 1.7 , 6.7 ± 1.5 and 7.9 ± 1.1 min, respectively to recognize the objects that was not significantly different from control values ($T1=3.5 \pm 0.3$ min, $P>0.05$) (Fig. 1).

At the second trial, RI for morphine with-drawn animals receiving vehicle was $-24.1\% \pm 6.6$ that was significantly different from normal values ($RI=45.8\% \pm 7.5$, $P<0.05$). By using spironolactone 50 and 100 mg/kg, RI improved to $10.8\% \pm 6.0$ and $24.0\% \pm 6.1$, respectively that was significantly different from the vehicle

treated animals ($P<0.05$) but also it differed significantly from normal values ($P<0.05$) (Fig. 2).

To evaluate the effect of spironolactone on memory performance in the object recognition task, spironolactone 100 mg/kg was used in normal animals and the results were compared with vehicle. RI in animals that received spironolactone was $45.8\% \pm 7.5$ which was not different from control group ($RI=45.8\% \pm 6.7$, $n=6$, $P=0.9$).

Spontaneous withdrawal

Based on our previous studies 4 h withdrawal was considered as the best time for this part of the experiment, since learning was not impaired and the effects of morphine withdrawal on memory were obvious in our experimental condition (26).

In the first trial, in accordance to our previous findings, 4 h after the last dose of morphine duration of T1 in morphine dependent animals receiving vehicle or spironolactone 50 and 100 mg/kg were 6.2 ± 1.1 , 6.8 ± 1.0 and 4.6 ± 1.1 min, respectively which dose not show significant difference from control values ($T1=2.2 \pm 0.2$ min, $P>0.05$) (Fig. 3).

RI for morphine dependent animals undergoing 4 h withdrawal receiving 50 mg/kg spironolactone was $18\% \pm 13.0$ that differed significantly from vehicle treated animals ($RI=-20.8\% \pm 11.4$, $P<0.05$) (Fig. 4). However, RI for dependent animals receiving 100 mg/kg spironolactone was $3.3\% \pm 11.9$, that was not different from vehicle and it differed significantly from control values ($RI=45.8\% \pm 6.7$, $P<0.05$).

DISCUSSION

As we have reported previously, glucocorticoid concentration increased following withdrawal from morphine (26), and by using mifepristone and metyrapone morphine withdrawal induced memory impairment was reversed. Since both MRs

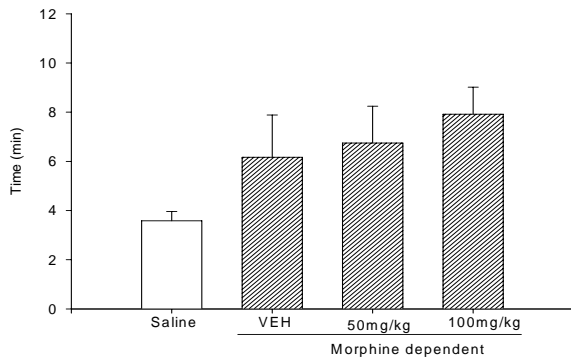


Fig. 1. Effect of spironolactone on time required for 20 s object recognition in the first trial (T1) in morphine dependent mice. In all groups n=6, results are expressed as mean ± S.E.M.

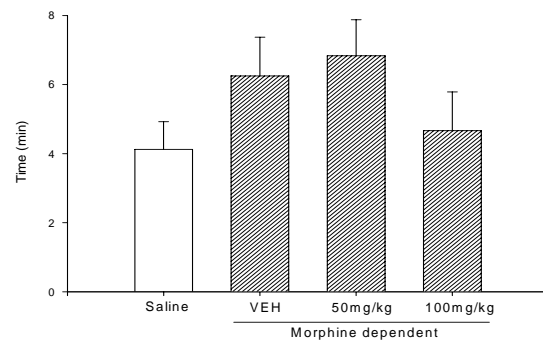


Fig. 3. Effect of spironolactone on time required for 20 s object recognition in the first trial (T1) in morphine dependent mice 4 h after the last dose of morphine (spontaneous withdrawal). In all groups n=6, results are expressed as mean ± S.E.M.

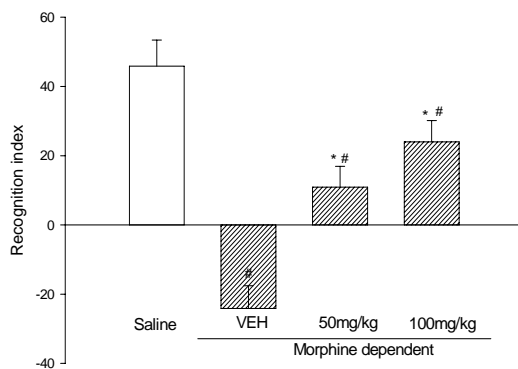


Fig. 2. Effect of different doses of spironolactone on memory performance (expressed as recognition index) in the two trial object recognition task after naloxone-precipitated morphine withdrawal. In all groups n=6, results are expressed as mean ± S.E.M. * $P < 0.05$ compared with vehicle values, # $P < 0.05$ compared with saline values.

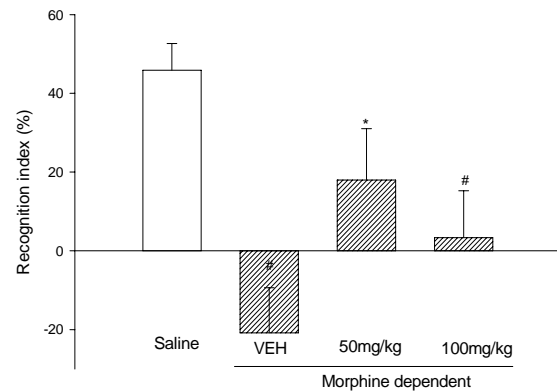


Fig. 4. Effect of different doses of spironolactone on memory performance (expressed as recognition index) in the two trial object recognition task after spontaneous morphine withdrawal. In all groups n=6, results are expressed as mean ± S.E.M. * $P < 0.05$ compared with vehicle values, # $P < 0.05$ compared with saline values.

and GRs complement each other, in the present study, the effect of spironolactone was determined on recognition memory following morphine withdrawal.

The object recognition task allows a rapid evaluation of memory performance in mice (31). In contrast to studies of memory in human subjects, animal experiments generally use emotionally arousing learning tasks. But in this method no rewarding or aversive stimulation is used during training, the learning occurs under condition of relatively low stress or arousal (32). This method has been

previously shown to be less strain-dependent (33).

In order to evaluate the validity of this memory paradigm, we examined the effect of scopolamine on the performance of mice in the object recognition task (Data not shown). In agreement with the results of other studies on the object recognition task (28,34), a single injection of 0.5 mg/kg scopolamine administered 10 min before T1 caused amnesia in mice.

Naloxone at 0.1 mg/kg injected 3 h after the last dose of morphine caused withdrawal signs like penile grooming,

teeth chattering, few jumping and piloerection and as the RI indicate it also caused memory impairment (Fig. 2). As it is shown in fig. 1 and 3 duration of T1 in dependent animals receiving vehicle or spironolactone dose not differ from control values which indicate learning is not influenced in dependent animals in this experimental condition.

As it is shown in fig. 2 and 4, at the second trial RI is very low (negative values) in naloxone precipitated and spontaneous morphine withdrawal animals receiving vehicle in the object recognition task. These animals can not discriminate between the new object and the familiar one, therefore memory performance is impaired. Using the object recognition task, spironolactone by its own did not have any effect on memory performance in normal animals. Therefore, its effect on memory after morphine withdrawal is due to its interactions with morphine withdrawal circumstances: like increased corticosterone concentrations.

It has been reported that the severity of naloxone precipitated morphine withdrawal in mice is more than spontaneous withdrawal and it can induce a server stress like state (35). Consequently, although spironolactone 50 and 100 mg/kg improved memory in mice after naloxone precipitated morphine withdrawal, memory impairment was not recovered (Fig. 2). This shows that even if spironolactone can improve recognition memory impairment following withdrawal but it can not overcome the stress load induced by naloxone.

By using spironolactone, mice undergoing spontaneous morphine withdrawal, improved memory retrieval in the object recognition task at a dose of 50 mg/kg and memory performance in these animals did not differ from normal values. As mentioned earlier, morphine withdrawal is associated with activation of the HPA (16). Previous reports showed that spontaneous

morphine withdrawal in mice is not very intensive and it would cause mild stress load (35). Therefore, in spontaneous morphine withdrawal animals' spironolactone either by antagonizing increased glucocorticoid effects on MRs and/or by nonspecific activity on GRs can improve memory impairment.

Under physiological condition, the degree of receptor occupation will range from a predominant MR occupation (under rest) to concurrent activation of MRs and GRs (at the circadian peak and after stress) (36). The balance in actions mediated by MRs and GRs in hippocampal neurons appears critical for neuronal excitability, stress responsiveness, and behavioral adaptation (36). Therefore, in our experimental condition in morphine-withdrawn animals, it is possible that, at least in part, spironolactone improved memory by balancing the activity of MRs and GRs in the hippocampus.

It has been suggested that escalating dose of morphine, can lower extracellular acetylcholine in brain areas, and during morphine withdrawal, acetylcholine release markedly increases (37). The role of the cholinergic system is well known in memory formation (38). Recent evidence suggests that testosterone can also modulate learning in males through an interaction with the cholinergic system (39). Findings suggest that testosterone may decrease the activity of the cholinergic system during non spatial tasks and thereby work in concert with the antagonism produced by scopolamine (39). Studies indicate that spironolactone is also an antagonist of the androgen receptor (40). Thus, it is interesting to state that some effects of spironolactone may be due to its inhibition on androgen system, and therefore by increasing the activity of cholinergic system it can improve memory performance during morphine withdrawal in the object recognition task.

Spironolactone binds to the MR with

much higher affinity than it does to the related GR (40). Since we have observed previously that mifepristone can improve memory deficit induced by morphine withdrawal in the object recognition task, the effects of spironolactone on memory is due to its non specific inhibition of GR to some extent,

In conclusion, spironolactone improves memory impairment following naloxone precipitated withdrawal, but it reverses memory performance to normal values in spontaneous withdrawal in our experimental condition indicating that it can not overcome the stress load induced by naloxone. Results of these experiments showed that not only the GRs but also MRs play an important role in recognition memory impairment following morphine withdrawal.

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