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Regular Article

Desmopressin accelerates the rate of urinary morphine excretion and attenuates withdrawal symptoms in rats

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Aim: The aim of this study was to examine the effects of desmopressin on morphine withdrawal symptoms and vasopressin level in morphine-dependent subjects.

Methods: Wistar male rats were injected s.c. with morphine once per day for 5 consecutive days to induce morphine dependence. After morphine use ceased on day 5, an equal number of rats were assigned to one of four groups for either saline or desmopressin by either intraperitoneal (i.p.) or intracerebroventricular (i.c.v.) injection. From days 5 to 10, urine was collected daily and tested for the presence of morphine, and withdrawal symptoms were monitored to assess the effects of desmopressin.

Results: Significant weight loss occurred among all morphine-addicted rats during the withdrawal period. With both methods (i.p. and i.c.v.), the period of urinary morphine excretion was shorter for the two groups that were given desmopressin (experimental groups) than the two groups that were not given desmopressin (control groups), and no significant difference in urinary morphine excretion was found between the two experimental groups. During the early stage of withdrawal, the severity of the withdrawal symptoms in the experimental groups was significantly lower than that in the control groups.

Conclusion: Desmopressin decreases the extent of morphine withdrawal symptoms, indicating that this agent might be appropriate for treating morphine addiction. Desmopressin appears to reduce withdrawal symptoms not by exerting an anti-diuretic effect but rather by exerting an effect on the central nervous system.

Key words: desmopressin, drug addiction, drug withdrawal, morphine, vasopressin.

DRUG ADDICTION AND withdrawal are problems throughout the world. Addiction not only affects the health of the individual but also causes problems for society, increasing the rates of divorce and unemployment and government expenditure on legal and medical systems. One particularly addictive substance is morphine, an aqueous solution that is primarily excreted from the body in urine.[1] Urinary excretion of morphine and conjugation of morphine in the liver leads to 83% of the drug being excreted from the body after the first day of use, with 11–14% of morphine later excreted in bile.[2] Renal clearance of morphine is known to occur by glomerular filtration and tubular secretion, and possibly also by reabsorption.[3]

In both humans and rats, the bilateral cationic transport system is responsible for morphine reabsorption and secretion,[3,4] with the kidneys playing an important role in 3-monoglucuronide and 6-glucuronide excretion, and possibly in normorphine excretion also.[5,6] Interestingly, i.v. 3-monoglucuronide morphine is excreted only by the kidneys.[2] After cessation of morphine use, rats

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have been shown to present withdrawal symptoms, including weight loss, pain tolerance reduction, tics, body and foot shaking, eyelid ptosis, and diarrhea.[7] Such morphine withdrawal symptoms can also be induced in morphine-dependent animals by naloxone, an opioid antagonist drug.[7] Daily and weekly repeated use of similar doses of morphine can have an additive effect on naloxone-precipitating withdrawal symptoms.[8]

Although morphine is known to increase levels of oxidant factors, it has also been reported to generate antioxidant activity. Research has found that powerful and efficient antioxidant activity can be generated with even small doses of morphine, and its antioxidant and alkali properties increase in a concentration-dependent manner.[9] In light of these findings, the role of antioxidants in morphine withdrawal symptoms has been thoroughly investigated. These investigations have demonstrated that the blood oxidative stress indices associated with the severity of morphine withdrawal symptoms increase with naloxone but can be reduced by an antioxidant agent.[7,10]

Given that withdrawal is known to induce stress, a major trigger for substance abuse,[11] it can lead to the resumption of drug use in a cycle of continuous substance abuse.[12] Common symptoms observed during the early phase of alcohol, cocaine, opiate, nicotine, and marijuana withdrawal include irritability, anxiety, emotional distress, sleep problems, dysphoria, aggressive behavior, and drug cravings.[13] The severity of these symptoms is known to predict the treatment outcome and the chance of relapse among smokers, cocaine and heroin addicts, and alcoholic subjects,[14,15] with research findings indicating that the greater the extent of dependence and abstinence, the greater the likelihood of relapse and poor treatment outcome.[14]

Physical stressors, such as chronic pain, have been observed to increase the level of vasopressin[16] in the rat amygdala and hypothalamus.[17,18] Vasopressin is an anti-diuretic, neurohypophyseal peptide hormone in mammals and is known to play a role in anxiety and stress responses.[18] One study on morphine withdrawal showed that withdrawal from acute, but surprisingly not chronic, cocaine consumption resulted in an increase in vasopressin mRNA level, which in turn resulted in an increase in the severity of early cocaine withdrawal symptoms.[19] Interestingly, that study also found that the severity of the symptoms could be reduced with naloxone.

While κ-opioid receptor agonists have been found to decrease serum vasopressin levels, leading to increases in urine output, other opioid receptors (μ and δ receptors) have been found to have little effect on reducing blood vasopressin levels.[20] Morphine has been observed to have dose-dependent and bilateral effects on the secretion of vasopressin: high doses stimulate vasopressin secretion and have provisional anti-diuretic effects and low doses lead to greater stability in inhibitory effects of morphine.[21–23] Among the types of vasopressin receptors, vasopressin receptor type 1b (V1b) is known to have a role in the stress response. The hypothesis that V1b receptor blockage reduces the sensitivity of the adrenocorticotropic hormone (ACTH) response to stress[24] was confirmed in a study that measured levels of plasma ACTH and corticotropin-releasing hormone (CRH).[25,26]

Desmopressin (1-desamino-8-D-arginine vasopressin), a synthetic analogue of vasopressin and an agonist of the V1b and V2 receptors,[27] increases the permeability of renal tubules, thereby reducing urine volume. The strongest effects of desmopressin are observed after 6 h but all effects, including reduction in urine volume,[28] last up to 24 h. In healthy individuals, vasopressin increases the effects of CRH on ACTH production in the pituitary.[8] In alcoholic subjects, vasopressin reduces pituitary sensitivity to CRH, which could be a significant factor that strengthens the ACTH response to changes in CRH level.[26] Evidence indicates that the elevation of vasopressin resulting from morphine withdrawal results in increased levels of CRH and ACTH. Despite this evidence, there are no data supporting the role of the hypothalamic–pituitary–adrenal (HPA) axis in morphine withdrawal, nor regarding the effects of vasopressin and its analogues on morphine withdrawal symptoms. To fill this research gap, this study investigated the effects of desmopressin on morphine withdrawal symptoms in rats.

METHODS

This was a 10-day study conducted on 60-day-old male Wistar rats weighing 225–275 g. The rats were housed in groups of four per cage in an animal facility maintained at 22 ± 2°C under a 12-h light/dark cycle and given food and water ad libitum. All experimental protocols and procedures complied with the guidelines of the 1975 Declaration of Helsinki, as reflected in the guidelines of the Medical Ethics Committee, Ministry of Health, Iran, and were approved

by the Research and Ethics Committee of Urmia University of Medical Sciences.

Pilot study

In a pilot study conducted 3 weeks before the main experiment, naloxone (1 mg/kg) was injected s.c. on day 5 of morphine use, and the morphine withdrawal symptoms were assessed for 30 min. The pilot study was conducted only to confirm that morphine dependency had been induced, and no data were collected for statistical analysis.

Stereotaxic surgery

Stereotaxic surgery was performed before the initiation of morphine, on 12 of the experimental rats that would later be given either saline or desmopressin by intracerebroventricular (i.c.v.) injections. After the rats had been anesthetized by intraperitoneal (i.p.) injection of 50 mg/kg of pentobarbital (Merck, Darmstadt, Germany), the heads were shaved using a shaving machine (Moser, Unterkirnach, Germany) and secured in a stereotaxic apparatus. Under sterile conditions, a stainless steel cannula was implanted in the right lateral ventricle at a position 1.8 mm anteroposterior, 1.6 mm lateral, and 3.5 mm dorsoventral, according to the *Rat Brain Atlas* (Paxinos and Watson 6th edn). The guide cannula (N.23) was secured using two small steel screws and by applying dental acrylic on the skull bone. One cannula was placed into the guide cannula by a needle (N.30) to prevent cerebrospinal fluid secretion and obstruction of the cannula. Each rat received a single i.p. injection of cephazoline (120 mg/kg) in order to prevent infection. After a 5-day recovery period, the rats were divided into groups.

Formation and treatment of control and experimental groups

The primary study began with the formation of a control group (i.p.-S group) and an experimental group (morphine group). The control group consisted of eight rats given 0.5 mL of saline by i.p. injection twice daily for 5 consecutive days at 08.00 hours. The experimental group consisted of 24 rats, including the 12 that had undergone stereotaxic surgery. These rats were injected s.c. with additive doses of morphine sulfate solution (Temad Company, Tehran, Iran) once per day for 5 consecutive days (10, 15, 20, 30 and 30 mg/kg in 0.5 mL, respectively) at 08.00 hours. Although most previous studies had used higher doses of morphine (>30 mg/kg), we avoided such doses because they had resulted in considerable toxicity symptoms and mortality in the pilot study.

Urine collection and assessment

Urine samples were collected from the experimental group rats for evaluation of morphine levels, from 24 h after cessation of morphine use until 24 h after confirmation of morphine-negative samples. For collection and assessment, the experimental rats were placed in special cages between 08.00 hours and 12.00 hours daily, and the samples thereby obtained were stored at −20°C until morphine level could be assessed on thin layer chromatography (TLC).

Formation of experimental groups

After cessation of morphine on day 5, the experimental rats were divided into four groups ($n = 6$) and treated as follows: (i) morphine-i.p.-saline (M-i.p.-S) group: i.p. injection of 0.5 mL of saline twice daily for 5 days; (ii) morphine-i.c.v.-saline (M-i.c.v.-S) group: i.c.v. injection of 10 μL of saline twice daily for 5 days; (iii) morphine-i.c.v.-desmopressin (M-i.c.v.-D): i.c.v. injection of 1 μg/kg of desmopressin (10 μL) twice daily for 5 days; and (iv) morphine-i.p.-desmopressin (M-i.p.-D(group: i.p. injection of 12 μg/kg of desmopressin (0.5 mL) twice daily for 5 days.

Determination of total withdrawal score

For assessing behavioral symptoms of opioid withdrawal, the animals were studied individually in a clear Plexiglas chamber (50 cm × 25 cm × 15 cm) placed in a calm and noiseless room. A digital camera was connected to a recording computer attached to the chamber for simultaneous recording of the behavior. One observer blind to the treatment that the animals had received evaluated the reaction of each animal. The total withdrawal score was determined using the Mirzaii-Dizgah *et al.* modified method,[29] with recordings of behavior replayed for meticulous analysis, if necessary. Beginning 24 h after morphine cessation, three distinct withdrawal behaviors, namely, rearing, wet-dog shaking, and self-grooming, were scored on intensity during a

Table 1. Withdrawal symptom coefficients

	Withdrawal symptom		
	Rearing	Grooming	Wet-dog shaking
Coefficient	0.05	0.1	0.2

60-min session from 19.00 hours to 20.00 hours daily for 5 consecutive days. The score (count) for each behavior was multiplied by a coefficient (Table 1), and the resulting values were added to obtain a total withdrawal score for each animal.

Cannula placement confirmation

After completion of the behavioral testing, methylene blue (5 μL) was injected into the cannula of the cannulated rats, and the rats were killed using high doses of ether. After decapitation, the brain of each rat was extracted and dissected to confirm (i) proper placement of the cannula; and (ii) adequate distribution of methylene blue throughout the brain ventricles. Data were collected from rats that met these criteria, and were used for subsequent statistical analysis.

Statistical analysis

The three behavioral symptoms evaluated were assigned the coefficients shown in Table 1. Because the data for withdrawal score and bodyweight were found to have a normal distribution, 1-way ANOVA and Tukey's post hoc test were performed for analysis. Chi-squared tests were performed for assessing the urine morphine levels. All results are stated as mean ± SEM and were considered statically significant at $P < 0.05$.

RESULTS

Day 1 withdrawal scores

The withdrawal symptom scores are shown in Fig. 1. On day 1 after morphine cessation, no significant differences were detected between two of the experimental groups (M-i.p.-D and M-i.c.v.-D) and the control (i.p.-S) group. But a significant difference was detected between the addicted groups given desmopressin (M-i.p.-D/M-i.c.v.-D) and those given saline (M-i.p.-S/M-i.c.v.-S; $P < 0.001$). The withdrawal scores of the addicted groups given desmopressin (M-i.p.-D/M-i.c.v.-D) decreased considerably, approximating those of the i.p.-S group. Meanwhile, no significant differences were detected between the M-i.c.v.-D and M-i.p.-D groups, indicating that neither method of injection was superior to the other.

Day 2 withdrawal scores

On day 2 after morphine cessation, no significant differences were detected between the M-i.p.-S and M-i.p.-D groups ($P = 0.22$) or between the M-i.c.v.-S and M-i.c.v.-D groups ($P = 0.58$), indicating that neither method of desmopressin injection had a significant effect on morphine withdrawal symptoms. Based on the detection of significant differences between the i.p.-S group and the experimental groups (M-i.p.-D, M-i.c.v.-D, and M-i.p.-S, $P < 0.001$; M-i.c.v.-S, $P < 0.002$), we hypothesized that the withdrawal symptoms had increased with desmopressin use (as compared to day 1), indicating that desmopressin had no significant effect on morphine withdrawal symptoms on day 2.

Days 3–5 withdrawal scores

On day 3, desmopressin no longer had a significant effect on withdrawal symptoms, and this trend con-

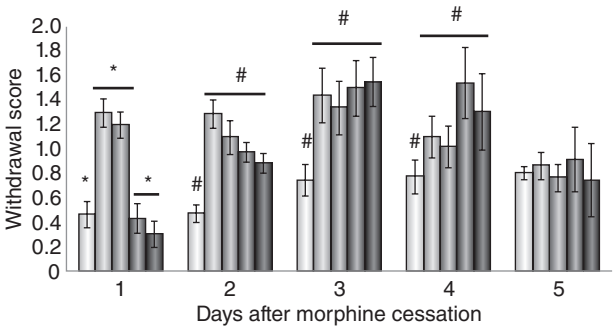

Figure 1. Effect of desmopressin on morphine withdrawal symptoms. Rats were injected with cumulative doses of morphine (M) for 5 days and tested for morphine withdrawal symptoms. Saline (S) or desmopressin (D) was injected either intraperitoneal (i.p.) or intracerebroventricular (i.c.v.), daily after morphine cessation. On day 1, the desmopressin groups (M-i.p.-D, M-i.c.v.-D) were similar to the control group (i.p.-S), while they were significantly different from the M-i.p.-S and M-i.c.v.-S groups (*$P < 0.001$). On days 2–4, desmopressin did not have any significant effect on withdrawal symptoms. #$P < 0.01$ between control and other groups. (□) i.p.-S; (▫) M-i.p.-S; (▫) M-i.c.v.-S; (▪) M-i.p.-D; (▪) M-i.c.v.-D.

Table 2. Urinary morphine level

Group Description	Group	Days after morphine cessation				
		Day 1	Day 2	Day 3	Day 4	Day 5
Control, receiving i.p. saline	i.p.-S	–	–	–	–	–
Morphine dependent, receiving i.p. saline	M-i.p.-S	+	+	+	±	–
Morphine dependent, receiving i.p. desmopressin	M-i.p.-D	+	+	+	–	–
Morphine dependent, receiving i.c.v. saline	M-i.c.v.-S	+	+	+	+	–
Morphine dependent, receiving i.c.v. desmopressin	M-i.c.v.-D	+	+	+	–	–

Presence of morphine in the urine was evaluated 24 h after morphine cessation. Rats receiving desmopressin (i.p. and i.c.v.) had negative urinary morphine tests by day 4 (chi-squared test, $P = 0.002$). i.c.v., intracerebroventricular; i.p., intraperitoneal.

tinued on days 4 and 5. These results indicate that desmopressin significantly attenuated withdrawal symptoms in morphine-dependent rats during the acute phase but had no significant effect during the chronic phase of withdrawal.

Effects of desmopressin on urinary morphine excretion

The results of daily evaluation of urine morphine level during the period of morphine withdrawal are listed in Table 2. While all morphine-treated groups had positive results on days 1, 2, and 3 after morphine cessation and negative results on day 5, the results obtained on day 4 varied considerably among the groups. These results suggest that desmopressin accelerated urinary morphine elimination, leading urinary morphine to be detectable for only a few days after cessation of morphine.

Effects of morphine withdrawal on bodyweight

All rats were weighed at the beginning of the study and subsequently weighed daily. No significant differences were detected in bodyweight among the experimental and control groups during the 5 days of morphine use. Although a progressive reduction in bodyweight was detected 2 days after morphine was commenced, this reduction was not significant. Although no significant differences in weight change were detected among the groups until morphine cessation, the four experimental groups tended to show significant weight loss compared to the control (i.p.-S) group on day 2 of morphine withdrawal, possibly because of stress from either experiencing withdrawal symptoms or undergoing tests. After day 3, the trend reversed, and all four experimental groups began regaining weight until they had regained all the weight they had lost (Fig. 2).

DISCUSSION

Addiction and withdrawal can have a significant impact on human society. Given that morphine dependency and the severe withdrawal symptoms associated with its cessation are stressors that tend to result in chronic morphine abuse,[12] identifying an effective means of relieving withdrawal symptoms is an important step in combating morphine addiction. Several studies have reported that vasopressin, a naturally occurring peptide hormone, is affected by morphine withdrawal,[21–23] but none examined the use of vasopressin to minimize withdrawal symptoms. To fill this research gap, this study examined the effect of desmopressin i.p. or i.c.v. injection on the withdrawal symptoms of morphine-dependent male rats. Because vasopressin is known to affect water excretion, urinary morphine excretion was also tested daily. The results indicate that desmopressin significantly reduced withdrawal symptoms on day 1 of morphine cessation but not on days 2–5.

Whereas several have reported that vasopressin levels increase early in withdrawal,[19,25] others have observed that elevated vasopressin leads to changes in HPA activity,[24,25] resulting in an increase in corticosteroid production. On the basis of these findings, it has been hypothesized that vasopressin could reduce withdrawal symptoms by exerting an unknown effect on corticosteroid production. More specifically, vasopressin is hypothesized to play a supportive role in combating the acute stress experienced during withdrawal by activating HPA activity

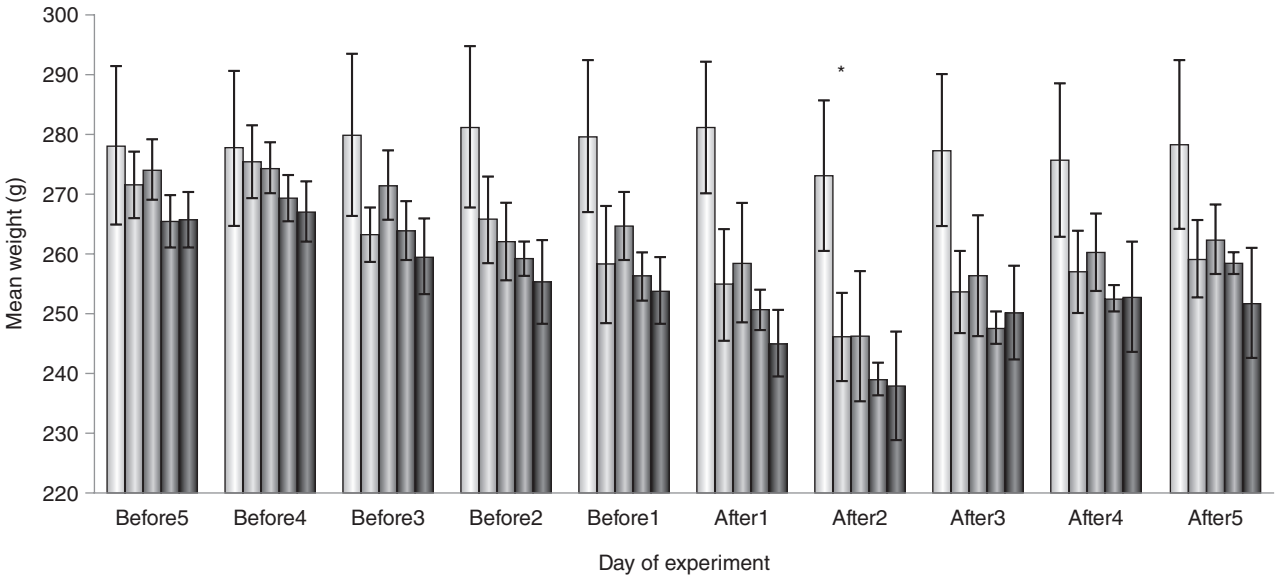

Figure 2. Effect of morphine withdrawal syndrome on rat bodyweight. After 5 days of morphine (M) in additive doses, morphine cessation significantly reduced bodyweight in rats. Weight reduction in addicted rats was significant ($P = 0.04$) 48 h after morphine cessation, which recovered by day 5 completely. D, desmopressin; i.c.v., intracerebroventricular; i.p., intraperitoneal; S, saline. (□) i.p.-S; (□) M-i.p.-S; (□) M-i.c.v.-S; (□) M-i.p.-D; (□) M-i.c.v.-D.

and glucocorticoid production,[24,25] both of which provide protection against stress.

The observation of increased vasopressin during the early stages of morphine withdrawal in previous studies is consistent with the observation that desmopressin attenuated withdrawal symptoms on day 1 of withdrawal in the present study. A growing body of evidence suggests that vasopressinergic neuronal activity in the amygdala and hypothalamus is an important element in the neurobiology of stress-related behaviors. Acute stress is known to increase extracellular levels of vasopressin in the rat amygdala and hypothalamus, and activation of V1b receptors by vasopressin has been observed to modulate anxiogenic and depressive behaviors in rats.[25] Of the three subtypes of vasopressin receptors – the V1a, V1b, and V2 receptors – the V2 receptor is located in the kidneys, where it mediates anti-diuretic action, while the V1a and V1b receptors are expressed in vascular smooth muscle[30] and various brain regions,[31] most precisely in the extended amygdala.[32] Recent investigations suggest that the stress-responsive AVP/V1b system may provide new therapeutic targets for preventing drug-abuse relapse.[32] It has been hypothesized that the vasopressin-V1b system may be a critical component of the neural circuitry underlying the aversive emotional consequences of drug withdrawal. In line with this hypothesis, SSR149415, a systemically active, selective V1b receptor antagonist, has been observed to significantly reduce foot-shock-induced reinstatement and block heroin-induced reinstatement in rats in a dose-dependent manner.[25] The results of recent investigations also suggest that the stress-responsive AVP/V1b system may provide new therapeutic targets for the prevention of drug relapse.[32]

Many studies have indicated that vasopressin is a primary factor in the regulation of anxiety and depression.[33,34,35] Among these studies, that of Ishizuka *et al*., which examined the effect of a selective serotonin re-uptake inhibitor (SSRI) and a serotonin-noradrenaline re-uptake inhibitor (SNRI), demonstrated that vasopressin V1b receptors are mediators for anti-anxiety drugs.[33] Such findings indicate that vasopressin plays a supportive role in combating acute stress by activating the HPA pathway.[24,25] Specifically, they suggest that stress and withdrawal lead to an increase in vasopressin, which in turn stimulates HPA activity and glucocorticoid production to help protect animals against stress.

Morphine has a biphasic, dose-dependent effect on vasopressin secretion,[21–23] stimulating vasopressin

secretion and exerting anti-diuretic effects at high doses (1–20 mg/kg). The anti-diuretic effect of morphine is not reduced by vasopressin antagonists, but is reduced by naloxone, an opioid antagonist.[34] At low doses, the inhibitory effect of morphine on vasopressin secretion is more stable, because it causes a reduction in plasma vasopressin level, which increases urinary morphine excretion.[22,23] It is hypothesized that an increase in morphine urinary excretion leads to a reduction in vasopressin level, increasing the severity of morphine withdrawal symptoms experienced by addicts. If this hypothesis is correct, then increasing the plasma vasopressin level would decrease the severity of morphine withdrawal symptoms.

Although desmopressin was observed to decrease withdrawal symptoms on day 1 of this study, it was not found to have a significant effect on urinary morphine excretion. These findings may have resulted from the central nervous system (CNS)-related effects of vasopressin interfering with the increased renal clearance of morphine or, in accordance with the findings of a previous study, the independence of the anti-diuretic effect of morphine from the vasopressin pathway.[34] The most important renal mechanism in morphine excretion is glomerular filtration, with several studies reporting a direct relationship among the renal clearance of morphine, the urine flow rate (UFR), and the tubular reabsorption percentage (TR%). Because desmopressin increases water TR%, it may increase the rate of renal morphine clearance, as indicated by the fact that the urine morphine test results became negative over the course of this study. Moreover, a strong correlation has been reported between the rate of renal excretion of morphine and the glomerular filtration rate (GFR), with renal excretion having been shown to increase with serum morphine level.[3]

The observation of weight loss after discontinuation of morphine (Fig. 2) is consistent with reports indicating that most weight loss occurs 48 h after the final morphine injection in morphine-dependent animals.[35] Weight loss is a primary factor to consider during early morphine withdrawal in rats, because it is indicative of physical dependence level.[35] Although desmopressin appeared to have had no effect on weight loss in the morphine-dependent rats examined in the present study, it may have reduced the severity of their morphine withdrawal symptoms. This possibility is supported by the finding that the rats given desmopressin had negative urine test results earlier than the control rats. In turn, this finding suggests that desmopressin does not decrease the severity of withdrawal symptoms by exerting an anti-diuretic effect but rather by exerting a CNS-related effect; thus, increased vasopressin level increases urinary morphine excretion rather than decreasing it. Further research evaluating urinary morphine concentrations, plasma vasopressin levels, and vasopressin concentrations in specific brain nuclei is necessary to examine this intriguing hypothesis.

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