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Effects of intracerebroventricularly-injected morphine on anxiety, memory retrieval and locomotor activity in rats: Involvement of vasopressinergic system and nitric oxide pathway

Nevzat Kahveci *, Guldal Gulec, Kasim Ozluk

Uludag University Medical School, Department of Physiology, 16059 Gorukle, Bursa, Turkey

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Abstract

Morphine has been shown to alter several behavioural processes. We aimed to investigate the effects of intracerebroventricular (i.c.v.) morphine on anxiety, memory retrieval and locomotor activity in rats and to elucidate the possible involvement of the vasopressinergic system and the nitric oxide (NO) pathway in these effects. Rats were pretreated with morphine (0.5, 5, 50 μg/5 μl; i.c.v.) or saline (5 μl; i.c.v.) 30 min before the elevated plus maze test, the probe trial of the Morris water maze and the open field test. Morphine (5 μg/5 μl; i.c.v.) induced significant anxiolytic effects in the elevated plus maze. None of the doses of morphine produced any effects in the probe trial of the Morris water maze and the open field. Pretreatment with an arginine vasopressin (AVP) V_1 receptor antagonist (25, 125 ng/5 μl; i.c.v.), an AVP V_2 receptor antagonist (25, 125 ng/5 μl; i.c.v.), or L-NAME, an NO synthase inhibitor (5, 25 μg/5 μl; i.c.v.) 30 min before morphine significantly prevented the anxiolytic effects of morphine. These results suggest that i.c.v. morphine has significant anxiolytic effects, probably mediated by both vasopressinergic system and NO pathway, but has no effect on memory retrieval or locomotor activity, at least at the applied doses.

Keywords: Morphine; Anxiety; Memory; Locomotor activity; Vasopressin; Nitric oxide

1. Introduction

Endogenous opioid peptides, the endorphins, dynorphins and enkephalins have a variety of functional effects in the organism including the regulation of mood, motivation and pain as well as involvement in respiration, gastrointestinal motility, endocrine and immune functions (Kieffer and Gaveriaux-Ruff, 2002; Peart et al., 2005). These effects are mediated by three major classes of G protein-coupled opioid receptors, mu, delta and kappa, whose activation inhibits adenylyl cyclase (Kieffer and Gaveriaux-Ruff, 2002; Peart et al., 2005). A large number of specific mu, delta and kappa agonists and antagonists have been developed and extensively studied to explore the distinct roles of these receptors.

Among these, morphine is a well-known alkaloid opiate which has a potent analgesic activity as well as undesirable effects, such as tolerance and physical dependence (Pasternak, 1993; Raehal and Bohn, 2005). Mu receptors are the main target sites for morphine binding, proven by the finding that the analgesic and addictive properties of morphine are abolished in mice which lack the mu-opioid receptor gene (Matthes et al., 1996).

It has been reported that both intraperitoneal (Shin et al., 2003; Zarrindast et al., 2005) and intraamygdaloid (Westbrook et al., 1997; Zarrindast et al., 2005) injections of morphine potently induce anxiolysis. On the other hand, Le Merrer et al. (2006) have demonstrated that microinjection of morphine into the lateral septum produces an anxiogenic-like effect, which can be reversed by pretreatment with the mu-opioid receptor antagonist naloxonazine.

The effects of morphine on cognition and behaviour have been widely investigated. Westbrook et al. (1997) have shown

* Corresponding author. Tel.: +90 224 4428514; fax: +90 224 4428832.
E-mail address: nevka@uludag.edu.tr (N. Kahveci).

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that injection of morphine into the nucleus accumbens impairs the fear conditioning to the context, implying the impairment of contextual learning in rats. Sala et al. (1994) have observed a long-term impairment of radial maze performance following both oral and intraperitoneal chronic morphine administration. Intraperitoneal injection of morphine has been shown to induce memory deficits in Morris water maze performance (Li et al., 2000, 2001) and in one trial passive avoidance task (Rezayof et al., 2006).

With locomotor effects of morphine being another point of interest, it has been put forward that peripheral injection of morphine induced dose-dependent effects on the locomotor activity, high doses increasing and low doses decreasing locomotion in the open field (Rezayof et al., 2006; Belknap et al., 1998; Patti et al., 2005). It has also been reported that microinjection of morphine into the ventral tegmental area (Bauco et al., 1993) or nucleus accumbens (Cunningham and Kelley, 1992b) dose-dependently produce an increase, or a decrease followed by an increase in locomotor activity. The hyperlocomotion has been suggested to be mediated through the increased dopaminergic activity in the mesocortical and mesolimbic pathways (Wise and Bozarth, 1987; Cano-Martinez et al., 2001), although contradicting data have also been reported (Kalivas et al., 1983; Vaccarino et al., 1986; Murphy et al., 2001).

It has been reported that vasopressinergic system may contribute to several effects of morphine, such as the development of tolerance to its antinociceptive (Yamashiro et al., 1990; McNamara and Skelton, 1992) and orexic (Gulati and Ray, 1995) effects. Both intracerebroventricular (i.c.v.) (Firemark and Weitzman, 1979) and intravenous (Wilkens and Yates, 2005) injections of morphine have been shown to increase plasma arginine vasopressin (AVP) levels, leading us to hypothesize that AVP might mediate behavioural effects of morphine. Similarly, contribution of nitric oxide (NO) in several behavioural effects of morphine including memory formation and state dependent-learning (Khavandgar et al., 2003), conditioned place preference (Kivastik et al., 1996; Zarrindast et al., 2002) and morphine self administration (Sahraei et al., 2004) has previously been reported. Shin et al. (2003) have shown that L-arginine inhibits and L-NAME enhances the anxiolytic effect of acute morphine in mice. Finally, morphine-induced hyperactivity has been shown to be increased by L-arginine and attenuated by L-NAME (Zarrindast et al., 2003).

The i.c.v. route of drug administration may offer advantages for the treatment of various diseases, especially when the efficient brain concentrations cannot be reached due to the peripheral distribution and metabolism of the drug. In addition, systemic toxicity may be avoided, obtaining high intracerebral drug concentrations while reaching low plasma levels (Harbaugh et al., 1988; Barcia et al., 1999). In fact, i.c.v. route has already been used for morphine infusion for intractable cancer pain (Weigl et al., 1987). Yet, there is no adequate data about the effects of i.c.v. morphine administration on behaviour. Here, we aimed to investigate the effects of i.c.v. morphine on anxiety, memory retrieval and locomotor activity in rats, and also to elucidate the possible involvement of the vasopressinergic system and the NO pathway in these effects.

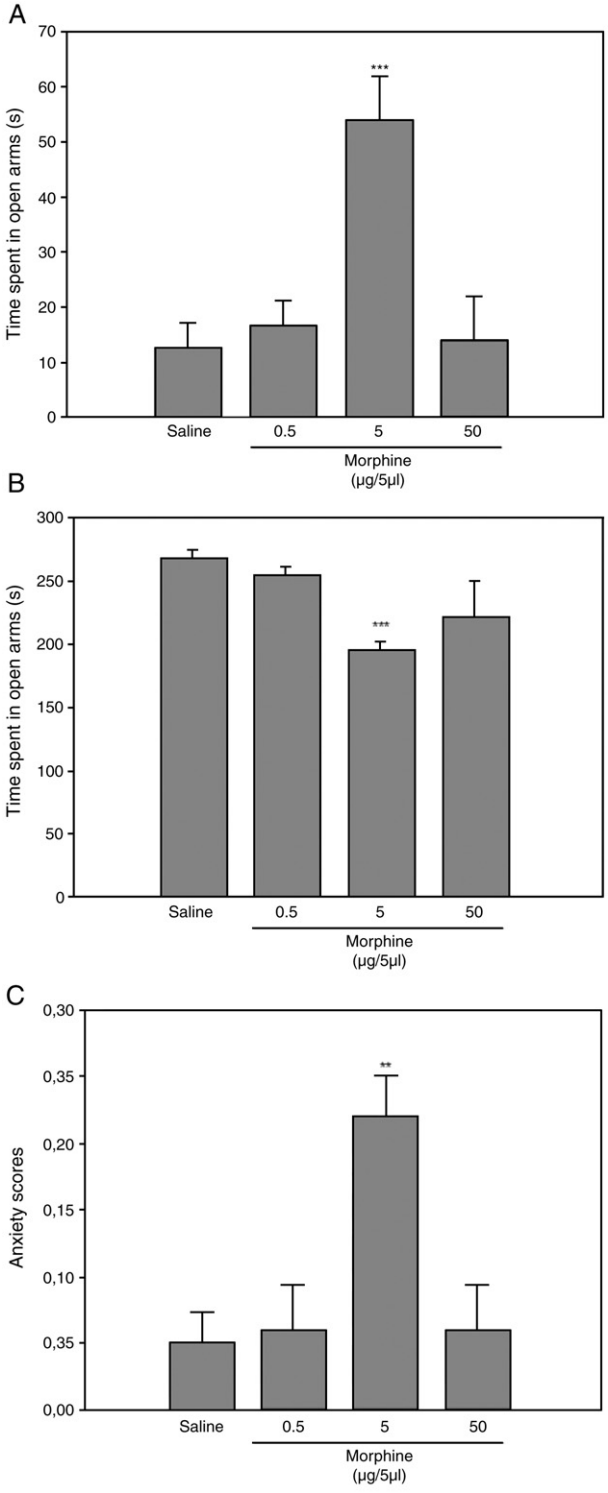

Fig. 1. Effect of i.c.v. morphine on anxiety behaviour. Rats received morphine (0.5, 5, 50 μg/5 μl; i.c.v.) or saline (5 μl; i.c.v.) 30 min before the elevated plus maze test. Time spent in open arms (A), time spent in closed arms (B), and the anxiety scores (time spent in open arms/time spent in open+closed arms) (C) were evaluated. Results were presented as means±S.E.M. Each group consisted of 6–7 rats. $^{**}p<0.01$ and $^{***}p<0.001$ with respect to the saline-treated rats.

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats, weighing 300–350 g were obtained from Uludag University Experimental Animals Breeding and Research Centre and were housed 4–6 in a cage with food and water available ad libitum. The colony room was maintained at 20–24 °C with a 12-h light–dark cycle. The surgical and experimental protocols used were approved by the Animals Care and Use Committee of Uludag University. All treatments were in accordance with the National Institutes of Health Guide of the Care and Use of Laboratory Animals.

2.2. Surgery

For i.c.v. injections, each rat was equipped with a permanent cannula. Under ether anesthesia, a burr hole was drilled through the skull 1.5 mm lateral to the midline and 1–1.5 mm posterior to bregma on the right side. Through this hole, a 10 mm length of 20 gauge stainless steel hypodermic tubing was directed toward the right lateral ventricle. The cannula was lowered 4.2–4.5 mm below the surface of the skull perpendicularly and was fixed to the skull with acrylic cement. Following the surgical procedure, rats were placed individually in cages and were allowed to recover from anesthesia for at least 4 h. During the recovery period, rats showed no evidence of pain.

2.3. Drugs

Morphine sulphate, AVP V_1 receptor antagonist ([Deamino-Pen^1, O-Me-Tyr^2, Arg^8]-vasopressin), AVP V_2 receptor antagonist ([Adamantaneacetyl^1, O-Et-$_D$-Tyr^2, Val^4, Amino-butyryl^6, Arg^{8,9}]-Vasopressin) and N^G-nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma Chemical Co. (St Louis) and dissolved in saline. I.c.v. injections were performed using a Hamilton microsyringe.

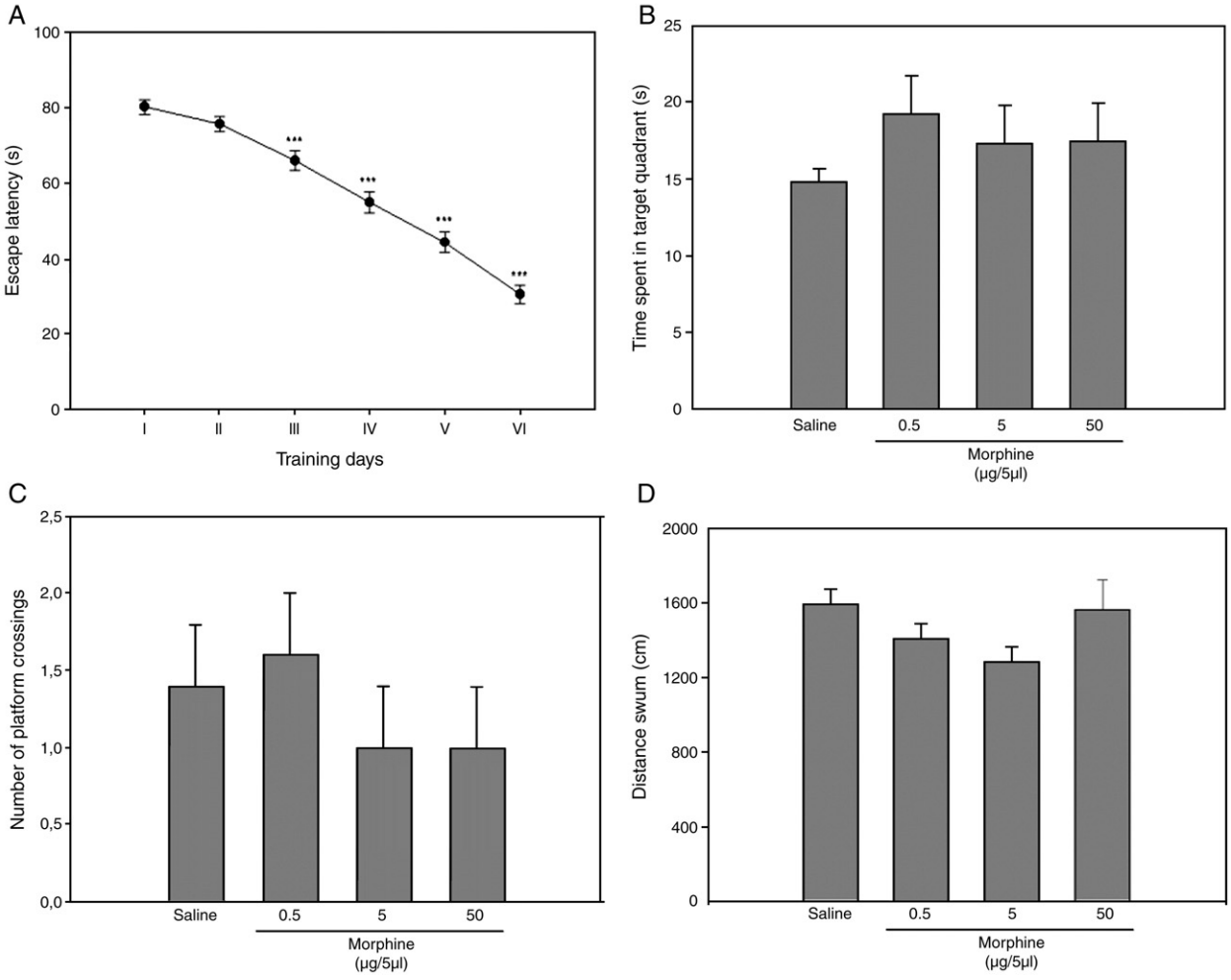

Fig. 2. Effect of i.c.v. morphine on memory retrieval. Rats were trained for 6 days in the Morris water maze to find the hidden platform. Escape latencies gradually decreased indicating that rats learned the task (***$p<0.001$) (A). On the 7th day, rats were treated with morphine (0.5, 5, 50 µg/5 µl; i.c.v.) or saline (5 µl; i.c.v.) 30 min before the probe trial. Time spent in the target quadrant (B), number of platform crossings (C) and distance swum (D) were observed. Results were presented as means±S.E.M. Each group consisted of 6–7 rats.

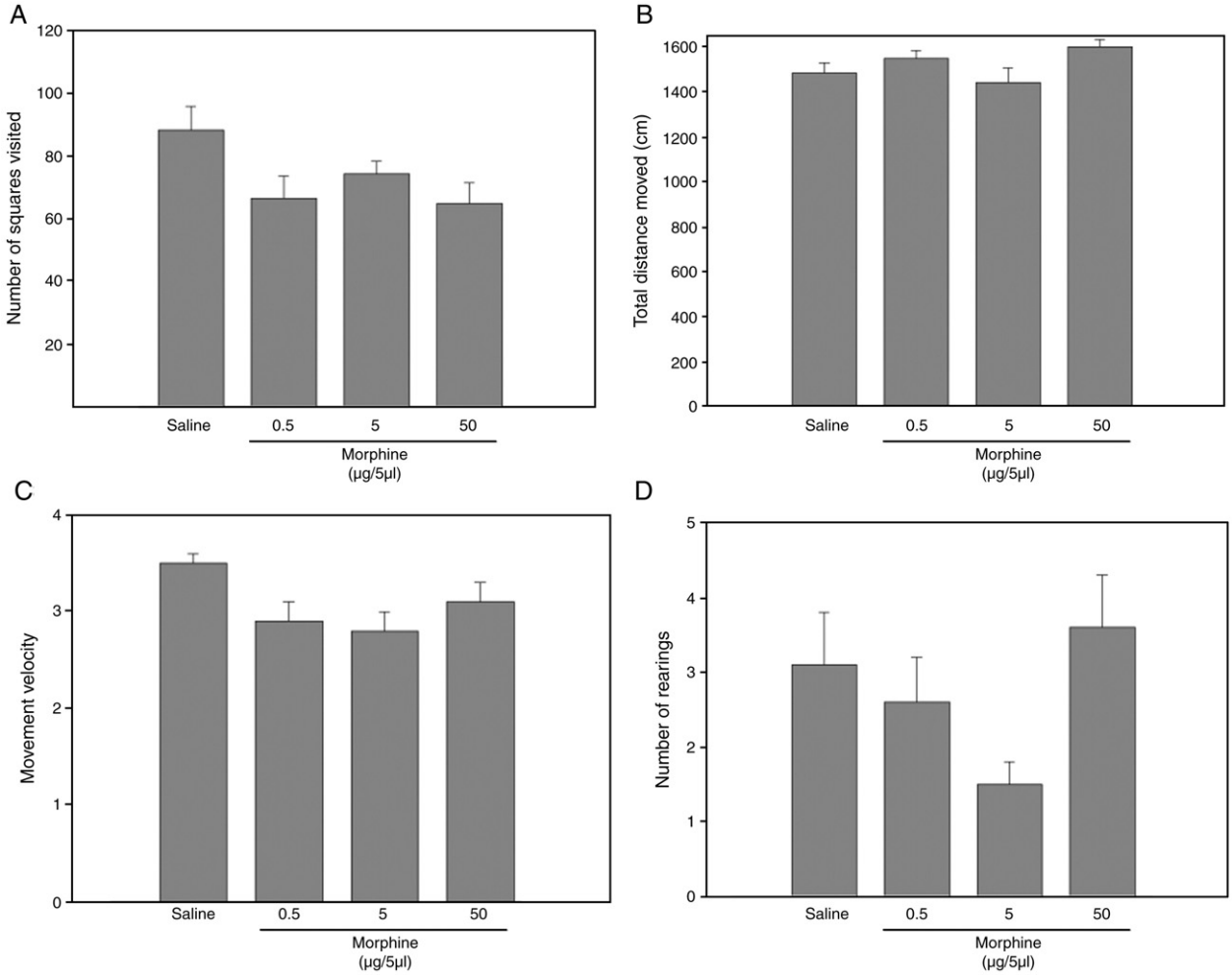

Fig. 3. Effect of i.c.v. morphine on locomotor activity and exploratory behaviour. Rats received morphine (0.5, 5, 50 μg/5 μl; i.c.v.) or saline (5 μl; i.c.v.) 30 min before the open field test. The number of squares visited (A), total distance moved (B), movement velocity (C) and the number of rearings (D) were recorded. Results were presented as means ± S.E.M. Each group consisted of 6–7 rats.

2.4. Behavioural tests

2.4.1. Elevated plus maze

Anxiety was evaluated in the elevated plus maze apparatus, which consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm) connected by a central square (10 × 10 cm) and was elevated to a height of 50 cm. The rat was placed in the centre of the elevated plus maze facing one of the open arms and was allowed to explore the maze for 5 min. The rat was considered to have entered an arm when all four limbs were inside the arm. After each trial, the apparatus was cleaned with 30% ethanol solution. Time spent in open arms, time spent in closed arms, and the ratio of the time spent in open arms to the time spent in open + closed arms (anxiety score) were measured.

2.4.2. Morris water maze

Spatial learning and memory were evaluated in the Morris water maze. The water maze consisted of a gray circular pool with a diameter of 150 cm and a wall height of 60 cm. The pool was filled with water at 25 ± 1 °C, to a depth of 30 cm. Four starting positions were located around the edge of the maze with equal distances, dividing it into four equal quadrants. A white escape platform (10 × 10 cm) was placed 1 cm below the water surface in the centre of one of the quadrants (the goal quadrant) and the water was made opaque by adding milk to make the platform invisible. The escape platform remained always in the same quadrant. Abundant extra-maze cues were provided with wall posters with geometrical figures, lamps and furniture.

Each rat received one training session consisting of four trials per day for six consecutive days. Four starting positions were randomly used in a session. During each trial, the rat was gently released from the starting position into the water, facing the pool wall. It was allowed to swim for 90 s to find the platform and to rest for 30 s on it. If it could not find the platform within 90 s, it was gently guided to the platform by hand and placed on it for 30 s. In the latter case, the latency to find the platform (escape latency) was accepted as 90 s. Having completed the fourth trial, the rat was gently dried and kept warm under a heat lamp before returning to its home cage. The mean of the escape latencies of the daily blocks of four trials were statistically evaluated.

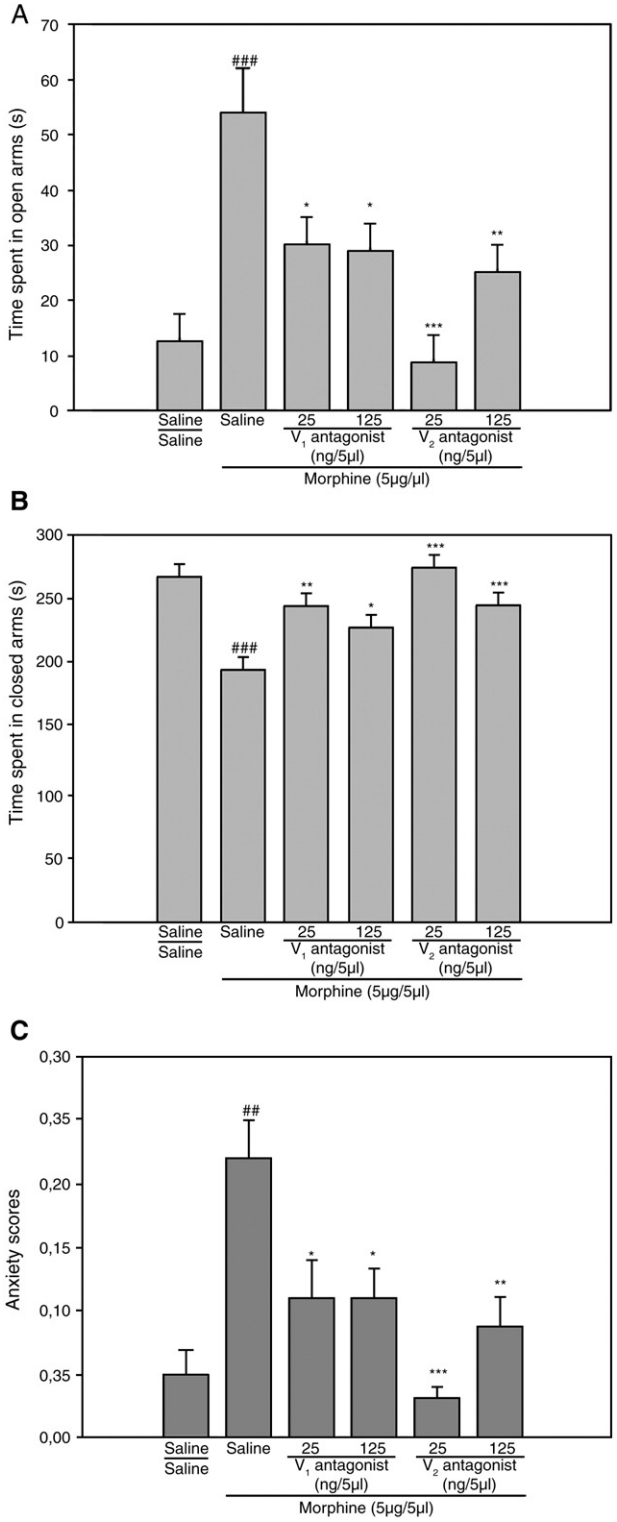

Fig. 4. Role of vasopressinergic system in the anxiolytic effect of i.c.v. morphine. Rats were pretreated with an AVP V_1 receptor antagonist (25, 125 ng/5 µl; i.c.v.) or an AVP V_2 receptor antagonist (25, 125 ng/5 µl; i.c.v.), 30 min before morphine (5 µg/5 µl; i.c.v.) injection. Elevated plus maze test was performed 30 min after morphine injection. Time spent in open arms (A), time spent in closed arms (B), and the anxiety scores (time spent in open arms/time spent in open+closed arms) (C) were evaluated. Results were presented as means±S.E.M. Each group consisted of 6–7 rats. $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ with respect to the saline+saline group. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ with respect to the saline+morphine group.

The probe trial was performed on the 7th day, when the platform was not placed in the pool and the rat was allowed to swim freely for 90 s. The time spent in the target quadrant, the number of platform crossings and the distance swum were measured to evaluate memory retrieval.

2.4.3. Open field

Locomotor activity and exploratory behaviour were measured in an open field area, which was a square plastic board (40×40 cm, walls 40 cm high) divided into 9 squares. The rat was placed into a corner of the area and was observed for 5 min. The number of squares entered by the four paws of the rat, total distance moved, movement velocity and the number of rearings were recorded.

All the behavioural test sessions were recorded by a CCD video camera mounted to the ceiling and all data were analyzed automatically by a computerized image analysis system, EthoVision (Noldus Information Technology, Netherlands) combined with the camera.

2.5. Experimental design

2.5.1. Experiment 1: effects of i.c.v. morphine on anxiety, memory retrieval and locomotor activity and exploratory behaviour

Rats were injected with morphine (0.5, 5, 50 µg/5 µl; i.c.v.) or saline (5 µl; i.c.v.) 30 min before undergoing the elevated plus maze test, the probe trial of the water maze test and the open field test.

2.5.2. Experiment 2: role of vasopressinergic system in the anxiolytic effect of i.c.v. morphine

Rats were pretreated with an AVP V_1 receptor antagonist (25, 125 ng/5 µl; i.c.v.) or an AVP V_2 receptor antagonist

Table 1
Effects of i.c.v. injections of V_1 receptor antagonist (25, 125 ng/5 µl), V_2 receptor antagonist (25, 125 ng/5 µl) and L-NAME (5, 25 µg/5 µl) 30 min prior to saline (5 µl; i.c.v.) on anxiety behaviour

	Time spent in open arms	Time spent in closed arms	Anxiety scores*
Saline+saline	12.7±2.6	267.6±8.0	0.04±0.01
V1 antagonist (25 ng/5 µl)+saline	26.6±8.8	263.2±17.9	0.09±0.01
V1 antagonist (125 ng/5 µl)+saline	29.6±4.5	242.7±13.4	0.10±0.04
V2 antagonist (25 ng/5 µl)+saline	17.8±3.6	254.2±18.9	0.06±0.01
V2 antagonist (125 ng/5 µl)+saline	16.3±5.2	253.5±5.1	0.06±0.02
L-NAME (5 µg/5 µl)+saline	27.2±7.8	249.8±13.1	0.09±0.02
L-NAME (25 µg/5 µl)+saline	16.2±0.6	257.3±15.2	0.05±0.01

Data were presented as means±S.E.M. Elevated plus maze test was performed 30 min after the second injection.
*Time spent in open arms/time spent in open+closed arms.

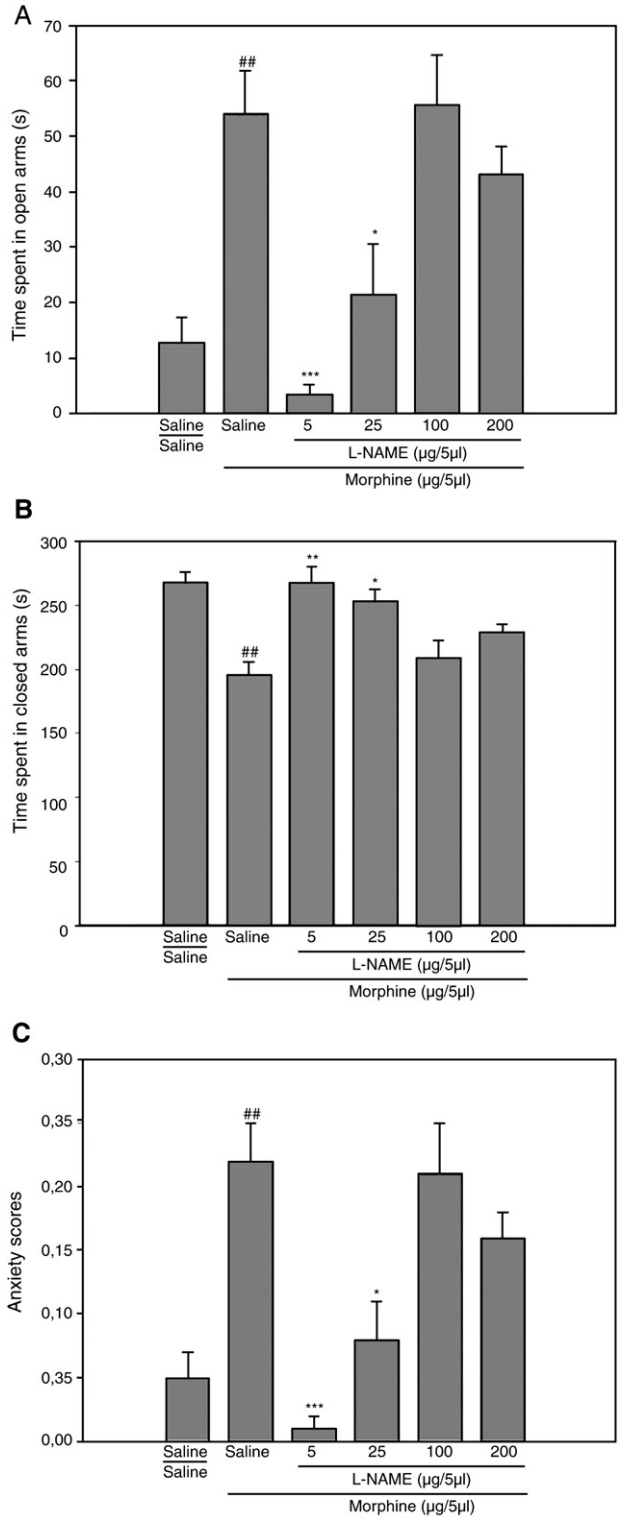

Fig. 5. Involvement of the NO pathway in the anxiolytic effect of i.c.v. morphine. Rats received N^G-nitro-L-arginine methyl ester (L-NAME), an NO synthase inhibitor (5,25,100,200 μg/5 μl; i.c.v.) 30 min prior to morphine (5 μg/5 μl; i.c.v.) injection. Elevated plus maze test was performed 30 min after morphine injection. Time spent in open arms (A), time spent in closed arms (B), and the anxiety scores (time spent in open arms/time spent in open+closed arms) (C) were evaluated. Results were presented as means±S.E.M. Each group consisted of 6–7 rats. $^{\#\#}p<0.01$ with respect to the saline+saline group. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ with respect to the saline+morphine group.

(25, 125 ng/5 μl; i.c.v.), 30 min before morphine (5 μg/5 μl; i.c.v.) injection. Elevated plus maze test was performed 30 min after morphine injection.

2.5.3. Experiment 3: involvement of the NO pathway in the anxiolytic effect of i.c.v. morphine

Rats received N^G-nitro-L-arginine methyl ester (L-NAME), an NO synthase inhibitor (5,25,100,200 μg/5 μl; i.c.v.), 30 min prior to morphine (5 μg/5 μl; i.c.v.) injection. Elevated plus maze test was performed 30 min after morphine injection.

At the end of the experiments, 5 μl of a methylene blue solution was injected into the cerebral ventricle through the cannula, to verify the placement of the inner end of the cannula. After decapitation, the brains were removed and sections were observed macroscopically to ascertain whether the cannula had been correctly placed into the lateral cerebral ventricle.

3. Statistical analysis

Data are presented as means±S.E.M. Analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons were used to determine statistical significance. Differences were considered to be significant at $p<0.05$.

4. Results

4.1. Experiment 1: effects of i.c.v. morphine on anxiety, memory retrieval and locomotor activity and exploratory behaviour

4.1.1. Elevated plus maze

The effects of i.c.v. morphine (0.5, 5, 50 μg/5 μl) on anxiety behaviour is shown in Fig. 1. Morphine at 5 μg/5 μl induced an increase in the time spent in open arms ($F(3,20)=10.90$, $p=0.0002$) (Fig. 1A), a decrease in the time spent in closed arms ($F(3,20)=6.31$, $p=0.0035$) (Fig. 1B), and an increase in the anxiety scores ($F(3,20)=11.61$, $p=0.0001$) (Fig. 1C). Higher and lower doses of i.c.v. morphine (0.5 and 50 μg/5 μl) did not seem to affect the anxiety behaviour in rats.

4.1.2. Morris water maze

Fig. 2A shows the significant decrease in mean latency to find the platform over the six training days ($F(5,230)=57.91$, $p<0.0001$), indicating that the rats learned the task. On day 7, rats were grouped randomly and were injected with different doses of morphine (0.5, 5, 50 μg/5 μl; i.c.v) or saline (5 μl; i.c.v.), 30 min before the probe trial was performed. As shown in Fig. 2B–D, there were no significant differences in the time spent in the target quadrant, the number of platform crossings and the distance swum between the saline-treated and the morphine-treated groups.

4.1.3. Open field

None of the applied doses of i.c.v. morphine (0.5, 5, 50 μg/5 μl) produced statistically significant differences in the number of squares visited, total distance moved and movement velocity (three measures of locomotor activity) or number of rearings

(measure of exploratory behaviour), compared to the saline-treated rats (Fig. 3).

4.2. Experiment 2: role of the vasopressinergic system in the anxiolytic effect of i.c.v. morphine

Fig. 4 illustrates the effects of pretreatment with an AVP V_1 receptor antagonist (25, 125 ng/5 μl; i.c.v.) and an AVP V_2 receptor antagonist (25, 125 ng/5 μl; i.c.v.) 30 min before morphine (5 μg/5 μl; i.c.v) injection. Both 25 ng/5 μl and 125 ng/5 μl doses of AVP V_1 receptor antagonist significantly abolished the anxiolytic effect of morphine, indicated by the decrease in the time spent in open arms ($F(5,34)=9.40$, $p<0.0001$), the increase in the time spent in closed arms ($F(5,34)=14.46$, $p<0.0001$) and the decrease in the anxiety scores ($F(5,34)=10.56$, $p<0.0001$) with respect to the saline+morphine group. Similarly, both doses of the AVP V_2 receptor antagonist inhibited the morphine-induced increase in the time spent in open arms ($F(5,34)=9.40$, $p<0.0001$), decrease in the time spent in closed arms ($F(5,34)=14.46$, $p<0.0001$) and increase in the anxiety scores ($F(5,34)=10.56$, $p<0.0001$). None of the antagonists alone had any significant effects on these parameters (Table 1).

4.3. Experiment 3: involvement of the NO pathway in the anxiolytic effect of i.c.v. morphine

I.c.v. injection of L-NAME (5, 25 μg/5 μl) 30 min prior to morphine (5 μg/5 μl; i.c.v.) also decreased the time spent in open arms ($F(5,25)=10.53$, $p<0.0001$) (Fig. 5A), increased the time spent in closed arms ($F(5,25)=9.27$, $p<0.0001$) (Fig. 5B) and decreased the anxiety scores ($F(5,25)=10.80$, $p<0.0001$) (Fig. 5C) with respect to the saline+morphine group (Fig. 5). Higher doses (100 and 200 μg/5 μl) of L-NAME were ineffective. L-NAME alone did not have any effect on these parameters (Table 1).

5. Discussion

In an attempt to investigate the behavioural effects of intracerebroventricularly-injected morphine, we used three validated behavioural measurement instruments, namely elevated plus maze, Morris water maze and open field, to assess anxiety, memory retrieval and locomotor activity and exploratory behaviour respectively, in rats.

Elevated plus maze, which was originally evaluated as a possible model of anxiety by Handley and Mithani (Handley and Mithani, 1984), is now reliably used to determine the anxiogenic and anxiolytic effects of drugs (Pellow et al., 1985; Pellow and File, 1986; Lister, 1987; Clement and Chapouthier, 1998). In the elevated plus maze, untreated rats spend more time in the closed arms due to their natural aversion for open spaces. Thus, anxiety is assessed by means of open arm avoidance and anxiolytic effect of a drug can be reliably expressed with the decreased aversion for open arms.

In our study, we found that i.c.v. injection of 5 μg/5 μl morphine induced anxiolytic effects in the elevated plus maze, as indicated by the increase in the time spent in open arms, decrease in the time spent in closed arms and the increase in the anxiety scores (ratio of the time spent in open arms to the time spent in open+closed arms). The anxiolytic action of morphine through various routes of injection has previously been reported. Both subcutaneous (Koks et al., 1999; Shin et al., 2003) and intraperitoneal (Zarrindast et al., 2005) injections of morphine have been shown to increase the number of open arm entries and time spent in open arms. Motta and Brandao (1993) have reported that microinjection of morphine to the dorsal periaqueductal gray induces antiaversive effects in low doses, which can be reversed by systemic injection of naloxone in doses that block mu-opioid receptors. In addition, Anseloni et al. (1999) have shown that higher doses of morphine injection to the dorsal periaqueductal gray induces aversive effects, which can be inhibited by a selective inhibitor of kappa opioid receptors, suggesting the dual activity of morphine in the dorsal periaqueductal gray via different opioid receptors. Authors have also demonstrated the involvement of dorsal periaqueductal gray in the antiaversive effects of low dose morphine.

Data about the mechanisms involved in the anxiolytic action of morphine are limited. Koks et al. (1999) have reported that cholecystokinin (CCK) B receptors may be involved in the anxiolytic effect of morphine, since an agonist of CCK_B receptors BOC–CCK-4 completely reversed the action of morphine, and the combination of the subeffective dose of morphine with a CCK_B receptor antagonist L-365,260 potentiated the effect of morphine. Zarrindast et al. (2005) have investigated the involvement of the central histaminergic system in the morphine-induced anxiolysis and concluded that this effect might be probably independent of the histaminergic system. In the present study, we showed that anxiolysis induced by i.c.v. morphine might be mediated by both V_1 and V_2 receptors of AVP; since antagonism of both receptors significantly inihibited morphine's effect on anxiety. Involvement of the vasopressinergic system and especially the V_1 receptor in behaviour has been well-documented (Diamant and de Wied, 1993; Liebsch et al., 1996; Bielsky and Young, 2004; Hammock et al., 2005; Bielsky et al., 2005), whereas the role of AVP in behavioural effects of morphine has not been widely investigated. Yamashiro et al. (1990) have shown that daily pretreatment with i.c.v. anti-AVP antiserum suppresses the development of tolerance to morphine. In addition, blockade of the development of morphine tolerance by footshock and psychological stress was dose-dependently abolished by daily i.c.v. injections of AVP. The antagonistic effect of AVP on the tolerance development to the orexic effect of morphine has also been demonstrated (Gulati and Ray, 1995). Recently, it has been reported that intravenous injection of morphine significantly increases plasma AVP concentrations in ferrets (Wilkens and Yates, 2005). Milanes et al. (1997) have shown that acute and chronic morphine administration induce AVP increase in different brain nuclei, suggesting a complex response to morphine. Appenrodt et al. (1998) have demonstrated the anxiolytic effects of both centrally and peripherally applied AVP. Thus, morphine might exert its anxiolytic effect, at least in part, through an increase in AVP levels. However, the interaction

between morphine and the vasopressinergic system needs to be further elucidated.

We also demonstrated that inhibition of NO synthesis by L-NAME significantly inhibited the anxiolytic effect of i.c.v. morphine, suggesting that NO may mediate the central anxiolytic effect of morphine. In contrast to our results, Shin et al. (2003) have demonstrated the enhancement of the anxiolytic effect of acute morphine by L-NAME in mice. The discrepancy may be explained with the differences in animal species and also with the peripheral route of drug administration used, suggesting that mediation of anxiolytic effect of morphine by central NO may use different pathways. Although it has been shown that, L-NAME itself also produces significant anxiolysis in the plus maze (Faria et al., 1997), in the present study L-NAME alone did not modify the anxiety parameters at the applied doses.

It has been shown that hippocampal CA3 mu-opioid receptors play an important role in spatial learning and memory (Meilandt et al., 2004). A number of studies have demonstrated the spatial learning deficits induced by morphine administration in the Morris water maze (McNamara and Skelton, 1992; Li et al., 2000, 2001), but the effects of morphine on memory retrieval has not been assessed in the water maze. However, it has been shown that, pre-training systemic administration of morphine induces impairment of memory formation in different paradigms (Izquierdo, 1979; Bruins Slot and Colpaert, 1999; Khavandgar et al., 2002). There is also growing data about the morphine-induced state-dependent learning. It has been reported that impairment of memory formation induced by acute pre-training morphine injection can be reversed by pretesting administration of the drug in a time- and dose-specific manner (Bruins Slot and Colpaert, 1999; Khavandgar et al., 2002, 2003; Patti et al., 2006). Bruins Slot and Colpaert (1999) have shown that in animals trained with morphine, recall of the response occurs only in the same morphine-induced state and in animals trained in the normal state, recall occurs in the same state but not in the morphine state. On the other hand, Patti et al. (2006) have found that mice trained after saline injection and received morphine only before the test session present similar or better retrieval when compared to the control mice. In the present study, the 90 s free-swim probe trial preceeded by various doses of i.c.v. morphine to evaluate the memory retrieval revealed that morphine had no effect at the applied doses on the retrieval performance. The different routes and time courses of drug administration as well as the different paradigms used may be responsible for the controversial data.

We could not observe an increase in locomotion and exploratory behaviour in i.c.v. morphine-treated rats, which were evaluated in the open field. In contrast, Zarrindast et al. (2003) have observed an increase in locomotor activity with high doses (20 and 50 mg/kg) of morphine, when administered intraperitoneally. Others have reported stimulant or depressive effects of morphine on locomotion depending on the dose and the interval after administration (Cunningham and Kelley, 1992a; Bauco et al., 1993; Belknap et al., 1998; Rodriguez-Arias et al., 2000; Patti et al., 2005). The controversial results are difficult to explain, but may be due to the dosage and route of morphine injection. Belknap et al. (1998) have suggested a genetic basis for the different responses to morphine in the open field, since they have observed large strain differences between mice from 15 standard inbred strains.

We conclude that intracerebroventricularly-injected morphine had significant anxiolytic effects, which seemed to be mediated by both vasopressinergic system and NO pathway, but had no effect on memory retrieval and locomotor activity.

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