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Original article

Telemetry monitoring for non-invasive assessment of changes in core temperature after spinal drug administration in freely moving rats

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ABSTRACT

Introduction: There are no data available about the effects of spinally administered drugs on thermoregulation in freely moving animals. The first goal of the present study was to throw light on the consequences of intrathecally administered saline as a vehicle on core temperature and motor activity in unrestrained conditions. The second goal was to characterize the effects of morphine on these parameters as a widely used antinociceptive drug in spinal anesthesia, and reveal the potential role of the N-methyl-D-aspartate (NMDA) receptors in these processes. **Methods:** For these purposes, male Wistar rats were catheterized intrathecally, and E-Mitter battery-free transponders were implanted intraabdominally to continuously monitor core temperature and locomotor activity. **Results:** Saline induced a short-lasting hyperactivity accompanied by significant and prolonged hyperthermia that was blunted by systemic paracetamol administration. Morphine had no impact on motor activity; however, it caused high but equivalent degree hyperthermia in a wide dose range (1–15 μg), suggesting that it reached its peak effect. In the highest applied dose (25 μg), the NMDA receptor antagonist kynurenic acid blunted the saline-induced hyperthermia, and all doses caused higher hyperactivity compared to vehicle or morphine injections. In combination, kynurenic acid significantly inhibited the morphine-induced hyperthermia. **Discussion:** These data suggest that this method might be a valuable tool for investigating the thermoregulatory and locomotor effects of different drugs at spinal level; however, the prolonged effects of intrathecal vehicle injections should also be considered. The results point out that morphine is a very potent hyperthermic drug that may act primarily on the efferent limb of thermoregulation, at least partially, via an indirect NMDA-receptor mediated action mechanism.

1. Introduction

The spinal cord is a crucial structure in thermoregulation, and it shares the attributes of thermosensitivity and integrative capacity with the anterior hypothalamus. Among other peripheral and central nervous system locations, the spinal cord also contains a population of thermoreceptors; furthermore, spinal sympathetic and somatomotor mechanisms have significant roles in thermogenesis as well (Morrison, Nakamura, & Madden, 2008).

Telemetry monitoring of conscious laboratory animals has been proven useful in the acquisition of reliable physiological data, and it has many advantages over conventional methods of data collection (Ilback, Siller, & Stalhandske, 2008; Jaszberenyi et al., 2009). This method enables the detection of core temperature together with gross motor activity in freely moving, undisturbed animals. Since one of the most important factors in determining the body temperature is motor activity; therefore, parallel recording of body temperature and locomotor activity is very important in detecting whether locomotor activity changes might have led to the temperature alterations after different interventions. To our knowledge, no study has been conducted so far to investigate the effects of intrathecally (IT) administered drugs (or even vehicles) on the core temperature together with locomotor activity in unrestrained conditions. Generally, the IT applied volumes of the drugs in rats are between 5 and 10 μL and an additional 10 μL vehicle is used to flush the catheter (Yaksh & Rudy, 1976). The primary aim of this study was to determine the effects of saline (as a vehicle) in a total volume of 15 μL on these parameters using telemetry (Respironics, Mini Mitter, VitalView, Oregon, USA).

There is little doubt that opioid receptors are involved in thermoregulation, and the preoptic anterior hypothalamus is generally considered

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to be the primary site of their action in this respect (Adler, Geller, Rosow, & Cochin, 1988; Baker & Meert, 2002; Xin, Geller, & Adler, 1997). Several human studies have reported on core temperature changes after spinal opioid administrations, but only a few animal studies have investigated the role of spinal opioid receptors in this respect (Dib, Cormareche-Leydier, & Cabanac, 1982; Martin & Naruse, 1982; Rudy & Yaksh, 1977; Ryan, Price, Warriner, & Choi, 2012; Smith, Welch, Dombrowski, & Dewey, 1993). These studies investigate restrained animals and/or the thermistor is introduced into the colon causing significant stress responses, which leads to modified thermoregulation and motor behavior (Allen et al., 2007; Fukazawa et al., 2005; Martin & Naruse, 1982; Rudy & Yaksh, 1977). Sympathetic preganglionic neurons activated by glutamate via NMDA receptors also influence the core temperature (LoPachin & Rudy, 1982, 1983; Rudy & Yaksh, 1977). The opioid receptor expressing spinal GABAergic interneurons can inhibit the discharge of these glutamatergic fibers (Morrison et al., 2008). Thus the secondary goal was to characterize the effects of IT administered morphine, the NMDA receptor antagonist, kynurenic acid, and their combination on core temperature and locomotor activity.

2. Materials and methods

2.1. Animals

After the approval was obtained from the Hungarian Ethical Committee for Animal Research (registration number: XIV/00761/2010), male Wistar rats were used for the experiments. Animals (age between 2 and 3 months) were kept under conditions of controlled temperature (22 ± 1 °C) and humidity (55 ± 10%) with a 06:00 to 18:00 light cycle and ad libitum water and food access.

2.2. Drugs

The applied drugs were ketamine hydrochloride (Calypsol, Richter Gedeon Rt., Budapest, Hungary), xylazine hydrochloride (CP-Xylazin, CP-Pharma, Burgdorf, Germany), gentamycin (Sanofi-Aventis, Budapest, Hungary), paracetamol (Fresenius Kabi, Budapest, Hungary), and morphine hydrochloride (Hungaropharma, Budapest, Hungary). Kynurenic acid (KYNA) was dissolved in 0.1 M NaOH, and the excess NaOH was back-titrated with 0.1 M HCl to a neutral pH and the volume was adjusted with physiological saline. All the other drugs were dissolved in physiological saline. In order to avoid the change in the temperature of the cerebrospinal fluid, the solutions were warmed to core temperature (37–37.5 °C) before the IT injections. Since there were no difference between the effects of the vehicle of KYNA and physiological saline, the latter was used as vehicle.

2.3. Surgeries

Rats were anesthetized with a mixture of ketamine hydrochloride and xylazine (72 and 8 mg/kg intraperitoneally, respectively). An IT catheter (PE-10 tubing; Intramedic, Clay Adams; Becton Dickinson; Parsippany, NJ; I.D. 0.28 mm; O.D. 0.61 mm) was inserted through the cisterna magna and passed 8.5 cm caudally into the subarachnoid space (Yaksh & Rudy, 1976) to place the catheter tip between vertebrae Th12 and L2, corresponding to the spinal segments (L3–L6) that innervate the hindpaws (Dobos et al., 2003). The catheters were plugged to prevent infection through the cannula. The animals were also intraabdominally implanted with battery-free transponders to collect core temperature and locomotor activity data (E-Mitter, Philips Respironics®). A small lower abdominal incision was made, and a sterilized probe was inserted into the peritoneal cavity. Following the surgeries, the animals received antibiotic prophylaxis (10 mg/kg gentamycin, subcutaneously) to prevent infection, and at least a 4-day recovery (and washout for ketamine and xylazine) period was ensured. Rats exhibiting postoperative neurological deficits or not showing paralysis of one of the hindpaws after 100 μg IT lidocaine, which indicated the inappropriate location of the catheter, were excluded (about 10%) (Dobos et al., 2003). The animals were housed individually in cages of 40 × 30 × 18 cm that were placed on receiver platforms (ER-4000 Energizer Receiver) in an isolated room.

2.4. Acquisition

Body temperature and motor activity were monitored continuously for 24 h, from 2 h prior to IT injection to the beginning of the next light period. Regarding the activity monitoring, any change in signal strength from a transponder derived by the receiver was interpreted by the system as an indication that the transponder had moved and was scored as an activity count. With the transponder implanted into the peritoneal cavity, behavior involving finer movement occurs such as feeding or grooming without locomotion, was not registered as an activity. Instead, behaviors primarily including exploratory activity and ambulation were cumulated in activity counts (Harkin, O'Donnell, & Kelly, 2002). These counts were not suitable to quantify the magnitude or direction of movement. Sampling frequency regarding intraabdominal core temperature and general locomotor activity was set to 1 min throughout the experiment.

2.5. Experimental protocol

The animals were handled (they were taken out from their cage to stroke them for about 1 min) every day to reduce the emotional effects of the imminent experiments to a minimum. On the days of IT injections, besides the handling, the drugs were injected over 30 s. The cages were cleaned once a week. There was at least a 3-day wash-out period between the IT injections in a given rat (LoPachin & Rudy, 1982). Rats receiving a particular drug were started on a crossover design in which individual animals received IT injections of a control solution and of each of the designated drugs in different doses. To reduce the influence of diurnal core temperature changes, the injections were administered at the same time of the day. In the first series, the effects of IT saline in 15 μL of total volume were determined. Then the effects of intraperitoneally administered paracetamol (80 mg/kg) on the 15 μL saline-induced hyperthermia were investigated.

In the second series of experiments, the effects of morphine (0.1–15 μg), KYNA (1–25 μg) and their combination (1 and 10 μg, respectively) were determined. The volume of the drugs was 5 μL, and it was flushed with 10 μL saline (total volume of 15 μL). Since the volume of the catheter was about 6–7 μL, 10 μL saline flush ensured that the total volume of a drug could enter the IT space. The number of animals was 6 or 7 per treatment groups. At the end of the experiment, each animal was deeply anesthetized with an overdose of chloralhydrate.

2.6. Statistical analysis

To evaluate the prolonged effects of IT injections, data were averaged for each hour in the case of the core temperature, while motor activity was assessed as the summed count of activities per hour. To observe the acute effects of the injections on the core temperature, the mean of temperature data recorded every 10 min for the first hour after the injections were analyzed (based on a preliminary study), and the baseline value was calculated as the mean of values between 07:00 and 08:00 a.m. before the IT injection. Regarding the motor behavior for the first hour, the summed count of activities was calculated for 10-minute periods. Repeated measures ANOVA were used for the evaluation of the effects. Post hoc comparisons were carried out with the Fisher LSD test. Level of significance was set at $p < 0.05$.

3. Results

3.1. Effects of handling and vehicle injection on core temperature and motor activity

Body temperature and locomotor activity showed a clear-cut daily rhythm with night maxima and day minima characteristic of most rodent species.

Regarding the acute (1-h period) effects of the observed parameters after handling or IT saline injection, no significant differences were identified between the groups; that is both procedures caused a temporary enhancement in the motor activity accompanied by slight (0.6 °C) but significant hyperthermia (Fig. 1 A,B). Motor activity returned to the baseline level within one hour independently from the intervention (Fig. 1 B,D). Hyperthermia disappeared within two hours; however, the IT injection of 15 μL saline produced a second peak of hyperthermia with a higher increase (1.1 °C) that recovered only at the beginning of the dark period (Fig. 1 C). This intervention caused similar motor changes as the handling procedure during the light period, but significant hypoactivity was observed during the dark phase in this group (Fig. 1 D). As no other effects were observed during the dark period after any kind of treatment, only the data of the light periods were analyzed in further experiments.

Paracetamol (80 mg/kg, intraperitoneally) blunted the 15 μL saline-induced hyperthermia, without motor effects (Fig. 1 E). Repeated measures ANOVA showed significant effects of time ($F_{11,187} = 18.4$, $p < 0.001$), treatment ($F_{2,17} = 3.7$, $p < 0.05$), and time and treatment interaction ($F_{22,187} = 8.2$, $p < 0.001$) for core temperature during the 12-h light period.

3.2. Effects of morphine and KYNA

First the effects of different doses of morphine and KYNA were determined compared to the saline injection in the same total volume (15 μL).

The lowest dose of morphine (0.1 μg) did not cause any effect on abdominal temperature compared to the saline treatment; however, the 1 μg dose resulted in a peak hyperthermic effect; thus, the increase in dose (5 and 15 μg) did not result in further enhancement (Fig. 2 A, B). Morphine treatment did not influence motor activity as compared to saline injection (data are not shown). The detailed analysis of the first hour showed that the hyperthermic effect of morphine appeared 10 min after the injection (Fig. 2 A). Repeated measures ANOVA revealed significant effects of treatment ($F_{429} = 8.6$, $p < 0.001$), time ($F_{6,174} = 94.7$, $p < 0.001$), and time and treatment interaction ($F_{24,174} = 5.3$, $p < 0.001$) for the first hour. Repeated measures ANOVA showed significant effects of time ($F_{11,319} = 91.5$, $p < 0.001$) and time and treatment interaction ($F_{44,319} = 3.9$, $p < 0.001$) for core temperature for the 12-h light period; the rise was about 1.8 °C and it lasted for approximately 4 h (Fig. 2 B).

Regarding the acute thermoregulatory effects of KYNA, repeated measures ANOVA showed significant effects of time ($F_{6,126} = 10.5$, $p < 0.001$) and time and treatment interaction ($F_{18,126} = 2.4$, $p < 0.005$) for the 1-h period. The highest dose produced blunted hyperthermia (Fig. 2 C). In contrast, enhanced motor activity was observed for 20 min after the injection of all doses (Fig. 2 D). Repeated measures ANOVA showed significant effects of time ($F_{6,126} = 29.4$, $p < 0.001$) and treatment ($F_{3,21} = 3.4$, $p < 0.05$). Regarding the long-lasting effects of KYNA, it did not influence the observed parameters significantly (data are not shown).

The combination of 10 μg KYNA (the dose that produced similar effect as saline treatment) with 1 μg morphine induced blunted hyperthermia compared to morphine alone (Fig. 3 A). For the first hour, repeated measures ANOVA showed significant effects of treatment ($F_{3,21} = 6.5$, $p < 0.005$), time ($F_{6,126} = 34.8$, $p < 0.001$), and time and treatment interaction ($F_{18,126} = 4.0$, $p < 0.001$). Repeated measures ANOVA also showed significant effects of time ($F_{11,231} = 40.6$, $p < 0.001$) and time and treatment interaction ($F_{33,231} = 2.8$, $p < 0.001$) throughout the 12-h light period (Fig. 3 C). However, it was observed again that application of KYNA alone and in combination led to hyperactivity in the first hour as compared to saline or morphine (Fig. 3 B). Repeated measures ANOVA showed significant effect of time ($F_{6,126} = 16.2$, $p < 0.001$) only in the first hour. As for the diurnal motor activity, no significant differences were observed between these groups (data are not shown).

4. Discussion

Telemetry monitoring of conscious laboratory animals has been proven useful in the acquisition of reliable physiological data and has many advantages over conventional data collecting techniques (Ilback et al., 2008; Jaszberenyi et al., 2009). To our knowledge, this is the first study to investigate the effects of IT injections on core temperature and motor activity in freely moving animals, showing prolonged hyperthermic effects of IT injection of saline in a generally applied volume (15 μL). Furthermore, a high degree of hyperthermia was observed after morphine administration, even in very low doses, with no effect on the motor activity. The NMDA antagonist, KYNA, by itself did not cause long-lasting changes in these parameters, but it inhibited the morphine-induced hyperthermia.

Temporary but significant effects of handling were detected in both of the observed parameters with no influence on the daily rhythm of the animals. While the acute effects of IT saline injection did not differ from the effects of handling, the prolonged hyperthermia is heretofore not described phenomenon after vehicle injections. To fully explain such an effect of saline injection on core temperature is very difficult, but it can be hypothesized that the IT injection led to an inflammatory process leading to hyperthermia, since this sign was blunted by the non-steroidal anti-inflammatory drug, paracetamol. The other potential explanation might be due to the effects of changes in osmolarity after the injection of physiological saline heated to body temperature. However, in the case of solutions with low concentration (below 500 mM), the body temperature changes are negligible (Chawla, 2014); therefore, we suppose that a small change in the osmolarity should not have led to the observed very prolonged hyperthermia.

We did not find any effect on the locomotor activity after morphine administration. This is in contrast with the study of Martin, Zhang, Buechler, Conklin, and Eisenach (2005), who found that IT morphine at 1 or 3 μg dose reversed the inhibitory effects of IT catheterization on motor behaviors, while the higher dose of morphine (10 μg) significantly decreased the distance traveled, ambulation, stereotypy and rearing of the animals. It is supposed that the differences between the applied methods for the observation of the motor behavior might give an explanation for these differences.

Regarding the thermoregulatory properties of opioids in humans at spinal level, there are controversial results; studies reported both hypo- and hyper-thermic effects in antinociceptive doses (Kurz, Sessler, Schroeder, & Kurz, 1993; Ryan et al., 2012; Segal, 2010). Originally Rudy and Yaksh (1977) have investigated the thermoregulatory effects of spinally administered morphine in restrained rats, where the thermistor has been introduced into the colon causing significant stress for the animals (Rudy & Yaksh, 1977). In their study, morphine has caused dose-dependent hyperthermia (2–45 μg); 2 μg of morphine has caused only slight hyperthermia, while 15 μg has produced about 1 °C increase in colon temperature. In contrast, in this study, morphine induced higher degree of hyperthermia in freely moving rats, and it reached its peak effect (about 1.8 °C increase) at 1 μg, a dose without antinociceptive properties (Martin & Naruse, 1982). Another study has showed that morphine caused a U-shape dose-response curve (11.25–90 μg), where the lower doses have resulted in higher degree of hyperthermia (Martin & Naruse, 1982), but other studies have found that IT morphine did not influence the rectal temperature in

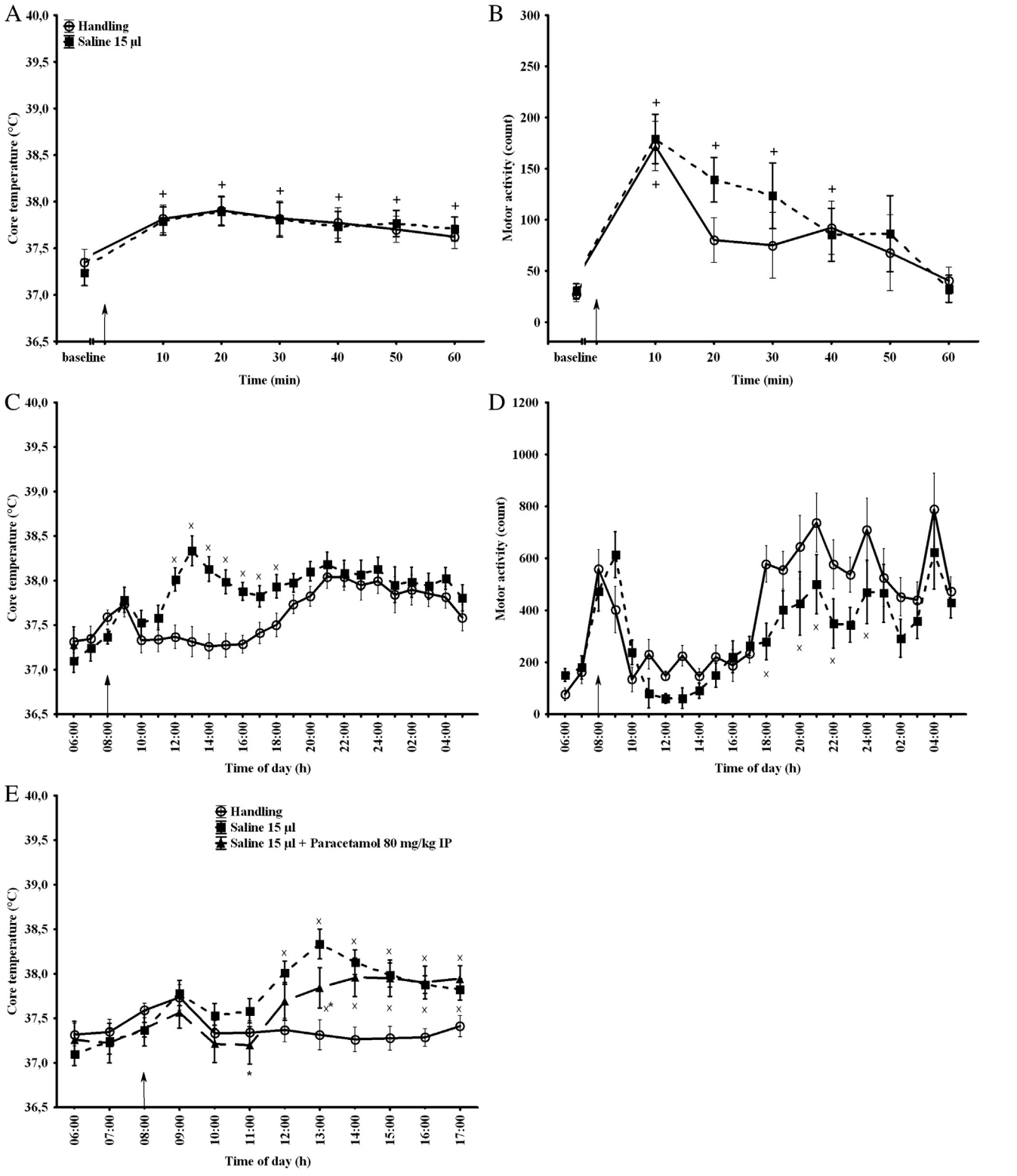

Fig. 1. Acute effects of handling or IT injection of 15 µL saline on abdominal core temperature (A) and locomotor activity (B) within the first hour. Diurnal rhythm of the observed parameters throughout a day (C, D). The effect of intraperitoneal paracetamol on IT saline-induced changes of abdominal core temperature (E) during the light period. Data are shown as mean ± SEM. The arrow signs the time point of handling or injections. The symbols indicate significant differences compared to the baseline value (+), handling procedure (X) or IT saline injection (*).

rats (10 µg) or in dogs (170 µg) (Allen et al., 2007; Fukazawa et al., 2005). These data indicate that the applied doses, the route of administration or the level of stress may greatly affect thermoregulatory responses to opioids.

There are data to suggest that the NMDA receptors also play a role in thermoregulation. Systemic administration of ketamine (5–100 mg/kg) produces dose-dependent hypothermia in rats, which is due to increased skin temperature caused by vasodilation and decreased metabolic heat production (Lin, Chen, & Pang, 1978). In contrast, another study, using telemetry, has found no significant effects of ketamine in similar dose ranges at different ambient temperatures in rats, but a subanesthetic (25 mg/kg) dose has enhanced the motor activity

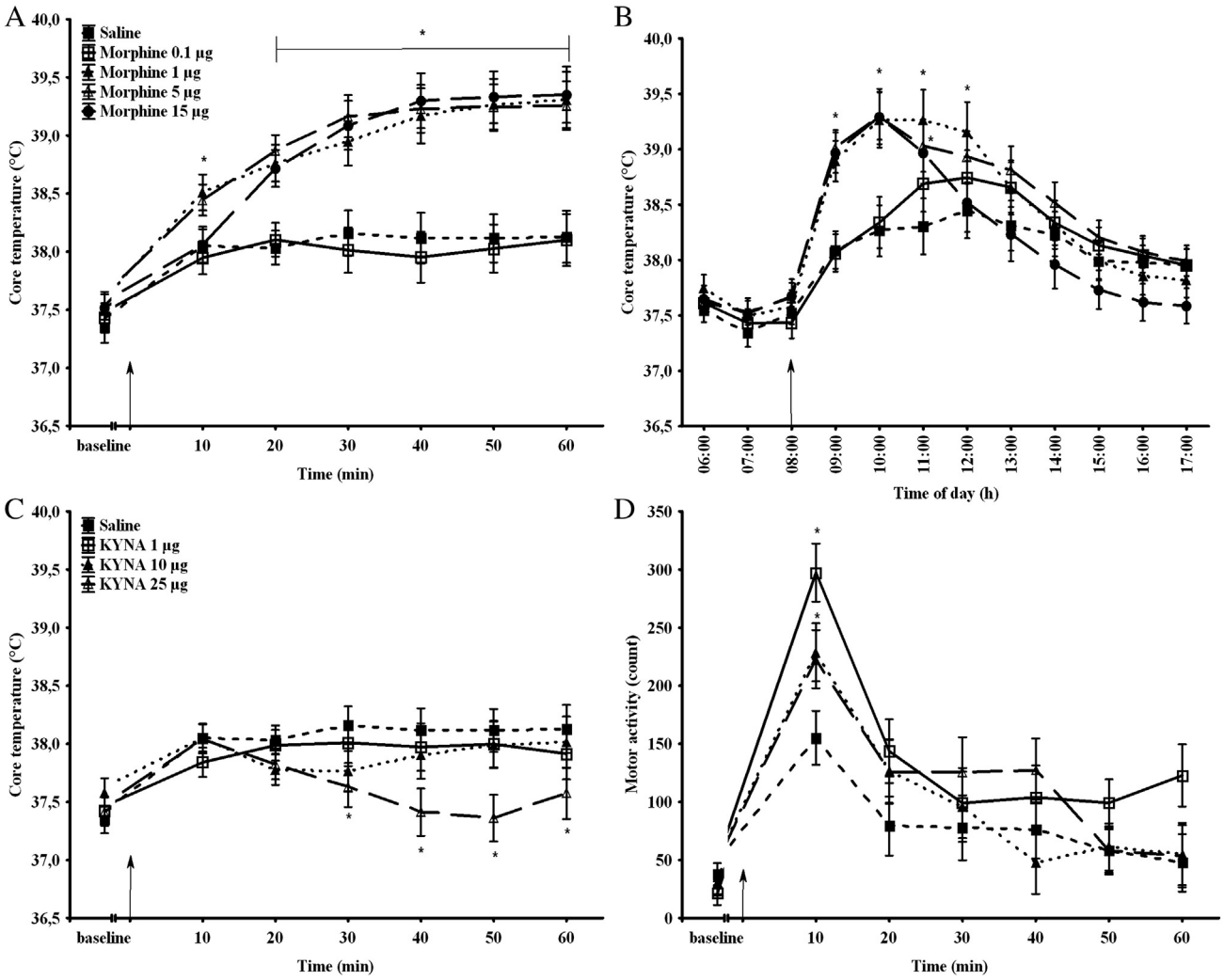

Fig. 2. Changes in the abdominal core temperature during the first hour after IT drug administrations (A). The dose- and time-dependent effects of IT morphine on thermoregulation (B) during the light period. Dose-dependent acute effects of IT KYNA on abdominal core temperature (C) and motor activity during the first hour after IT injections (D). Data are shown as mean ± SEM. The arrow signs the time point of IT injection. The symbol * indicates significant differences compared to IT saline treatment.

(Barbier et al., 2012). Regarding the role of NMDA receptors at spinal level, it has been shown that IT injection of NMDA increases the sympathetic nerve activities supplying skin circulation and the brown adipose tissue (Gilbey, 2007; Madden & Morrison, 2008); however, spinal administration of ketamine to dromedary camels and goats has not changed the core temperature (Azari, Molaei, Emadi, Sakhaee, & Esmaeili, 2012; Kinjavdekar, Amarpal, Aithal, & Pawde, 2007). Recent results have clearly showed that the endogenous NMDA receptor antagonist, KYNA, administered alone did not induce profound and long-lasting effects on body temperature and motor activity at the applied doses, but the highest dose caused a temporary decrease in core temperature. Regarding the motor activity, all doses of KYNA (alone and in combination with morphine) caused short-lasting hyperactivity compared to vehicle or morphine injections.

There are several mechanisms by which drugs can alter core temperature. One possibility might be that the effect is mediated by motor activity, a parameter known to have a considerable effect on core temperature. A prolonged hyperthermic effect of morphine was found, which was not accompanied by hyperactivity, suggesting that the observed hyperthermia could not be explained by increased spontaneous locomotor activity. In agreement with this result, an earlier study also suggests that the effects of systemic opioids on core temperature do not correlate with motor activity (Adler et al., 1988). An enhanced skeletal muscle tone can also lead to enhanced core temperature, and it is well-known that systemic administration of high doses of opioids leads to muscle rigidity (Weinger et al., 1995), while moderate doses decrease the muscle tone (Struppler, Burgmayer, Ochs, & Pfeiffer, 1983). Although the skeletal muscle tone was not recorded, the above mentioned results suggest that the morphine-induced hyperthermia in these low doses is presumably not due to enhanced muscle tone. The original study of Rudy and Yaksh shows that the hyperthermic effect of IT morphine is not antagonized by systemic indomethacin excluding inflammatory processes, but IT administered naloxone reduces this effect of systemically administered morphine, suggesting that the spinal opioid receptors are involved in the thermogenic effects of morphine (Rudy & Yaksh, 1977). Altered activity of sympathetic preganglionic neurons innervating the skin and the brown adipose tissue may be another important factor that can influence core temperature (LoPachin & Rudy, 1982, 1983; Rudy & Yaksh, 1977). It has been shown that the IT injection of morphine is associated with a decrease in skin temperature, which suggests active vasoconstriction (Rudy & Yaksh, 1977). In contrast, noradrenaline and clonidine induce hypothermia with increased skin temperature, together with a decreased activity of neurons in sympathetic ganglia, while no changes in the muscle tone are observed (LoPachin & Rudy, 1983). Sympathetic preganglionic neurons are activated by glutamate acting on NMDA glutamate receptors, and spinal GABAergic interneurons can inhibit the discharge of these glutamatergic fibers leading to decreased glutamate release (Morrison et al.,

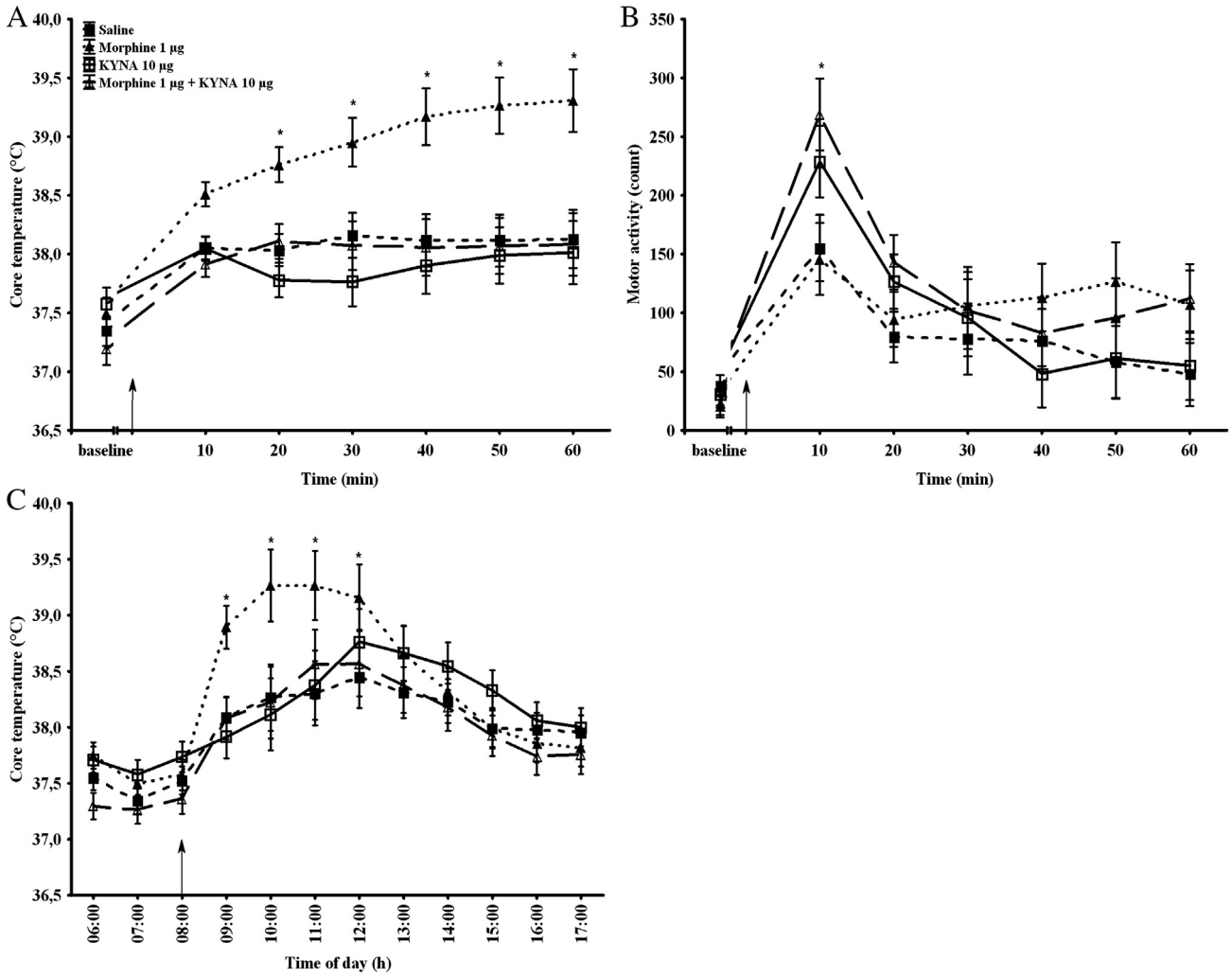

Fig. 3. The effect of IT KYNA (10 µg) on morphine-induced acute changes in abdominal core temperature (A) and locomotor activity (B). Thermoregulatory changes during the light period (C). Data are shown as mean ± SEM. The arrow signs the time point of IT injection. The symbol * indicates significant differences compared to IT saline treatment.

2008). It is noteworthy that some of the putative inhibitory inputs to these GABAergic neurons, i.e., through opioid receptor activation within the intermediolateral horn may provide a potential anatomical substrate for a pathway that increases the activity of sympathetic preganglionic neurons through disinhibition (Morrison et al., 2008). It is therefore tempting to suggest that morphine inhibits these GABAergic neurons, leading to the disinhibition of glutamatergic neurons that project on sympathetic preganglionic neurons. This hypothesis is supported by recent results, since KYNA inhibited the hyperthermic effects of morphine (10 µg, which itself did not modify the core temperature) possibly by the inhibition of the effects of the enhanced glutamate release (after morphine administration) on the NMDA receptors. Therefore, it can be proposed that effects on the thermoregulatory sympathetic efferents might lead to the observed changes of the core temperature after morphine administration. Since the co-administration of NMDA receptor antagonists and opioid receptor agonists can produce enhanced antinociception (Kekesi et al., 2002), they are antagonists in terms of their effect on the core temperature; thus, the combination of these ligands may be beneficial for pain therapy to prevent thermoregulatory side effects of morphine.

In conclusion, these data suggested that telemetry used in animals with chronic IT cannulation was a valuable tool for investigating the thermoregulatory and locomotor effects of different drugs at spinal level. These results showed significant effects of IT vehicle injection on body temperature that was inhibited by a non-steroidal anti-inflammatory drug. To our knowledge, these results are the first to demonstrate that very low doses of IT morphine cause long-lasting hyperthermia in freely moving animals. Furthermore, the inhibitory effect of KYNA on the morphine-induced hyperthermia suggests an indirect activation of NMDA receptors on sympathetic preganglionic neurons by morphine-induced disinhibition. It is supposed that this method might be appropriate for the preclinical assessment of the side effects of new, spinally administered drugs related to thermoregulation and/or motor activity.

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