Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 85 (2006) 292–297
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Conditioned place preference induced by morphine and morphine-6-glucuronide in mice☆

Vigdis Vindenes [a,*], Marte Handal [a], Åse Ripel [a], Fernando Boix [a], Jørg Mørland [a,b]

[a] Norwegian Institute of Public Health, Division of Forensic Toxicology and Drug Abuse, PO Box 4404, Nydalen, NO-0403 Oslo, Norway
[b] University of Oslo, The Medical Faculty, PO 1078-Blindern, NO-0316 Oslo, Norway

Received 22 March 2006; received in revised form 9 August 2006; accepted 17 August 2006
Available online 2 October 2006

Abstract

Morphine-6-glucuronide (M6G), an active metabolite of morphine has been shown to produce analgesia and fewer side effects than morphine, and the introduction of M6G as a new drug for treatment of postoperative pain is planned in 2007. Following morphine intake in humans, the metabolites morphine-3-glucuronide (M3G) and M6G are present in substantial concentrations and for longer periods than the parent drug. The possible reward effects of the morphine glucuronides have previously not been well studied. In the present study, conditioned place preference (CPP) was recorded after conditioning with subcutaneous injections of 5, 10, 20, 30 or 50 μmol/kg morphine or M6G, or 240 or 500 μmol/kg M3G in C57BL/6J-Bom mice, using a biased two compartment ("closed" and "open") counterbalanced paradigm.

CPP was induced after treatment with both morphine and M6G with dose dependent increase up to 30 μmol/kg after treatment in the "closed" compartment. No dose response was observed in the "open" compartment, with maximal CPP after 10 μmol/kg morphine or M6G.

M3G caused a tendency of condition place aversion (CPA), although not statistically significant. In the present study morphine and M6G demonstrated comparable reward effects, at doses that differed depending on which compartment the mice were conditioned in. M3G showed a tendency to exhibit aversive properties.

Keywords: Morphine-6-glucuronide; Morphine-3-glucuronide; Morphine; CPP; CPA; Reward

1. Introduction

Morphine is the most commonly used analgesic for severe pain. In humans morphine is conjugated by UDP-glucuronyltransferase in the liver primarily to morphine-3-glucuronide (M3G) and to a lesser extent to morphine-6-glucuronide (M6G), before elimination by the kidneys (Christrup, 1997). M3G and M6G are present in high concentrations for several hours after administration of morphine, even after the parent drug is no longer detectable, and could be of importance for the effects observed after morphine intake. M3G has no proven analgesic activity (Suzuki et al., 1993; Lipkowski et al., 1994), but its role in the analgesia of morphine is unclear. It has been reported that M3G antagonizes the antinociceptive effect of M6G (Gong et al., 1992), but other studies have shown no effect (Smith, 2000). One study has shown that co-injection of M3G and morphine increases and prolongs the analgesia caused by morphine (Lipkowski et al., 1994). The role of M6G in antinociception has been well studied, and it has been found to produce analgesia with a potency that equals or exceeds that of morphine (Christrup, 1997; Klepstad et al., 2000; Kilpatrick and Smith, 2005). M6G has recently been announced as a potential new drug for treatment of post-operative pain, with significant advantages over morphine and other opiates because of less nausea, vomiting and respiratory depression (Kilpatrick and Smith, 2005).

Opiates like morphine are widely abused, probably primarily because of their acute rewarding properties. The rewarding effect of morphine, which is well established, represents a disadvantage in therapeutic settings due to the potential for

☆ Parts of the results have been presented as a poster at the INRC in Annapolis 10–15 July 2005.

* Corresponding author. Tel.: +47 23 40 78 00; fax: +47 23 40 78 78.
E-mail address: vigdis.vindenes@fhi.no (V. Vindenes).

0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2006.08.010

misuse, and possible development of dependence and addiction. It is largely unknown how morphine glucuronides are related to reward. In light of the planned introduction of M6G in therapy (Kilpatrick and Smith, 2005), more information on its possible reward effects seem warranted.

Conditioned place preference (CPP) is a specific test for drug reward. It is thought to reflect a preference for a context due to the contiguous association between the context and a drug stimulus, and thus to represent the expression of experienced reward. The CPP paradigm has widely been used as an animal model of drug reward. A number of studies have investigated the reward effect of morphine, but M6G is not well studied. Mice metabolize morphine to M3G but not to M6G (Kuo et al., 1991; Dambisya et al., 1992; Christrup, 1997; Handal et al., 2002), which makes it possible to compare different effects of morphine and M6G.

We are not aware of previous studies where CPP was induced after systemic administration of M6G in mice, or studies on M3G in the CPP paradigm. The aim of the present study was to compare the effects of morphine, M6G and M3G in the CPP model in mice.

2. Materials and methods

2.1. Subject and apparatus

A total of 189 male C57BL/6J-Bom mice (from Bomholt, Denmark), weighing 19–24 g at testing, were used in the experiments. The animals were housed in standard plastic cages, containing small red shelters, for at least 5 days prior to the experiments. Food and water were available ad libitum, except during the behavioural tests. The colony room was illuminated with a 12 h light–dark cycle (light on from 07:00 am to 19:00 pm). Temperature in the room was 23±2 °C. The Norwegian Review Committee for the use of Animal Subjects approved the experimental protocol of this study.

Place preference and locomotor activity were measured by a Versamax optical animal activity monitoring system (AccuScan Instruments Inc., Columbus, USA). The cage size was 40×40 cm with infrared beams spacing 2.5 cm. Each cage was divided into two distinct compartments, connected by an opening that could be closed in the centre of the box. One compartment had black walls, a black ceiling and paper and sawdust as its floor, named "closed" compartment. The other compartment, named "open" compartment, had white walls, a transparent ceiling, and a metal plate with holes on the floor.

2.2. Drugs

Morphine hydrochloride (mol. weight 375.9) was purchased from Norsk Medisinaldepot (Oslo, Norway) and morphine-6-β-D-glucuronide dihydrate (mol. weight 461.47) and morphine-3-β-D-glucuronide (mol. weight 461.47) from Lipomed (Arlesheim, Switzerland). The drugs were dissolved in 0.9% saline (NaCl 0.9%). The injection volumes for morphine, M6G, M3G 240 μmol/kg and saline were 0.01 ml/gram mouse, and 0.02 ml/gram mouse for M3G 500 μmol/kg.

2.3. Treatment and procedure

The animals were injected subcutaneously (s.c.) about 1 cm cranial from the tail, with either morphine (5, 10, 20, 30 or 50 μmol/kg, respectively 1.9, 3.8, 7.5, 11.3 and 18.8 mg/kg), M6G (5, 10, 20, 30 or 50 μmol/kg, respectively 2.3, 4.6, 9.2, 13.8 and 23.1 mg/kg) or M3G (240 or 500 μmol/kg, respectively 110.8 and 230.7 mg/kg). The chosen doses of M3G were based on previous results from our group (Handal et al., in press), where the doses were well tolerated and did not alter locomotor activity. Control groups were injected only with physiological saline. The experiments were carried out during the light cycle. During each conditioning day (day 1–3) each mouse received one injection with drug and one with saline, with a 6 h interval. Immediately after the injections each mouse was carefully placed in one of the two compartments, which was closed off from the other in the test cage for 120 min conditioning, after which it was returned to the home cage. Half of the mice injected with active drug were conditioned in the "open" compartment, and the other half in the "closed" compartment. Half of the mice received saline as the first injection and the other half an opiate, every second day. The control group was injected with saline before being placed both in the "open" and the "closed" compartment, following the same schedule as the animals receiving active drugs. Half of the control group was defined as conditioned to the "open" and half to the "closed" compartment, before the study was started. At test day (day 4), after being injected with saline the mice were placed in the test chamber and had now for the first time free access to both the "open" and the "closed" compartments for 30 min. The time spent in each compartment was measured.

2.4. Statistical analyses

The time spent in the drug-paired compartment minus time spent in the unpaired compartment was used as a measure of preference for the drug-paired (conditioned) compartment, for the different doses of opiates, and compared to the saline group. A positive result was interpreted as CPP and a negative result as Conditioned Place Aversion (CPA).

Statistical differences were revealed by ANOVA, and Dunnett 2-sided test was used for post-hoc comparisons against saline. The time spent in "closed" versus "open" compartments for the saline group was compared using independent sample T-test. The distance travelled (cm/5 min) and total distance travelled (cm/120 min) on conditioning day 3 after treatment with different doses of morphine or M6G, were compared to the saline group using ANOVA and Dunnetts post-hoc test. P values off less than 0.05 were taken as statistically significant. Data are presented as mean±S.E.M. at each time point.

All the statistical analyses were conducted using the statistical package SPSS 12.0.1.

3. Results

Animals treated with morphine showed a significant CPP over the whole 30 min test session [$F(5, 85)=3.28$, $p=0.009$],

which was most evident during the first 10 min [$F(5, 85)=4.26$, $p=0.002$]. Dunnetts post hoc comparisons revealed that morphine doses of 10, 30 or 50 μmol/kg induced a significant CPP ($p<0.05$) after both 10 and 30 min test sessions, compared to the saline group (Fig. 1).

CPP was also seen for the animals treated with M6G compared to the saline group both after 10 [$F(5, 85)=2.33$, $p=0.049$] and 30 [$F(5, 85)=2.33$, $p=0.049$] minutes testing. Dunnetts post hoc comparisons indicated that M6G doses of 10 μmol/kg induced a significant CPP ($p<0.05$) during the first 10 min and doses of 10 and 30 μmol/kg when tested the whole 30 min (Fig. 1).

Statistically significant CPA was not seen for the animals treated with M3G compared to the saline group after 10 [$F(2, 45)=1.8$, $p=0.17$] or 30 [$F(2, 45)=0.60$, $p=0.55$] minutes, despite a clear tendency (Fig. 1).

Dose–response curves for CPP were different for the animals conditioned in the "closed" and "open" compartment (Fig. 2). Increase in CPP was seen for doses of morphine and M6G up to 30 μmol/kg in the "closed" compartment, with reduction for the higher dose of 50 μmol/kg. For the animals treated with drug in the "open" compartment, no association between the dose and the magnitude of CPP was observed, and maximum CPP was seen for morphine and M6G at doses of 10 μmol/kg. Animals conditioned in the "closed" compartment with morphine [$F(5, 39)=3.42$, $p=0.012$] and in the "open" compartment with M6G [$F(5, 40)=2.44$, $p=0.012$] showed statistically significant CPP.

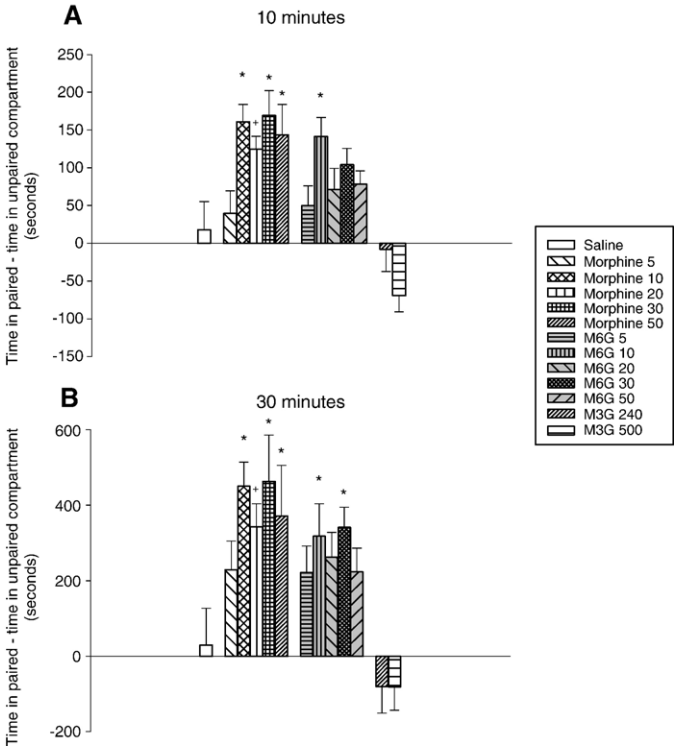

Fig. 1. CPP and CPA for the different doses (μmol/kg) of morphine, M6G and M3G compared to saline after 10 and 30 min. Bars ($n=14$ mice for the drug doses and $n=21$ for the saline group) represent mean time in paired compartment minus time in unpaired compartment (±S.E.M.). *$p<0.05$, +$p<0.10$.

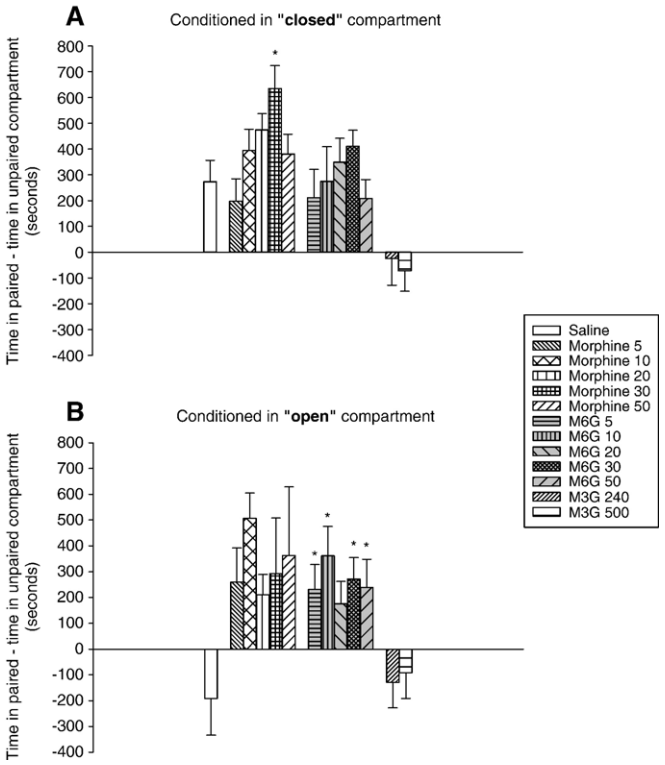

Fig. 2. CPP and CPA after conditioning in "closed" (2A) and "open" (2B) compartment after different doses (μmol/kg) of morphine, M6G and M3G compared to saline. Bars ($n=7$ mice for the drug doses and respectively 10/11 for the saline groups) represent mean time in paired compartment minus time in unpaired compartment (±S.E.M.) for 30 min on test day. *$p<0.05$.

Post hoc tests revealed that only 30 μmol/kg morphine in the "closed" compartment and 5, 10, 30 and 50 μmol/kg M6G in the "open" compartment induced statistically significant ($p<0.05$) CPP, although a clear tendency was seen for a several of the other doses.

An analysis of variance with dose and conditioned compartment ("open" vs. "closed") as factors showed a near significant effect of compartment [$F(1, 79)=3.84$, $p=0.053$] for the animals treated with morphine, but not for the interaction between both factors [$F(5, 79)=1.70$, $p=0.144$]. The animals treated with M6G exhibit a trend to an effect of compartment [$F(1, 79)=2.89$, $p=0.093$] and the interaction between dose and compartment [$F(5, 79)=2.13$, $p=0.071$]. A significant compartment effect was observed for M3G [$F(1, 42)=4.35$, $p=0.043$], but only a tendency for an interaction between both factors [$F(2, 42)=2.42$, $p=0.101$].

The saline treated animals showed preference for the "closed" compartment both after 10 ($p=0.009$) and 30 min ($p=0.013$) testing.

Dose–response curves, showing increased locomotor activity with increased doses, were seen for both morphine and M6G. Comparing the distance travelled revealed that morphine treatment significantly increased locomotor activity after 10 min [$F(5, 68)=24.46$, $p<0.001$] at 50 μmol/kg ($p<0.001$) and after

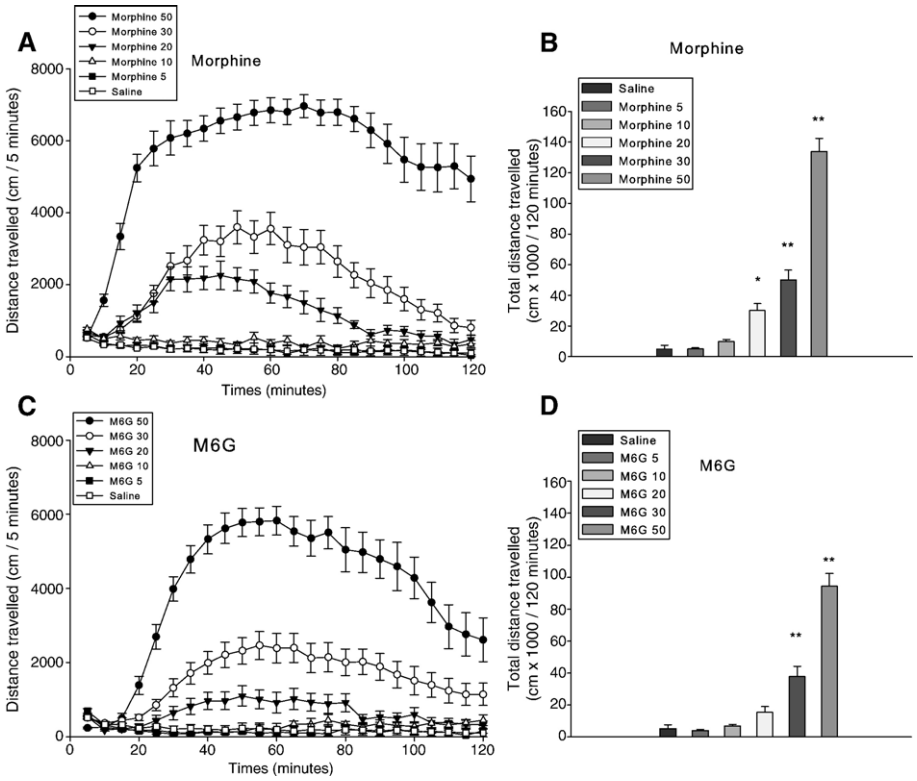

Fig. 3. Mean distance travelled (cm/5 min)±S.E.M. after s.c. injection with different doses (μmol/kg) of morphine (3A) and M6G (3C) on conditioning day 3, compared to saline. Mean total distance travelled (cm × 1000/120 min) for the different doses of morphine (3B) and M6G (3D) are illustrated as bars±S.E.M. *$p<0.05$ and **$p<0.001$.

20 min [$F(5, 68)=86.79$, $p<0.001$] at 20 μmol/kg ($p<0.05$) and 30 μmol/kg ($p<0.05$). After treatment with M6G, significantly increased locomotor activity was seen after 20 min [$F(5, 65)=18.78$, $p<0.001$] at 50 μmol/kg ($p<0.001$), and 30 min [$F(5, 65)=60.44$, $p<0.001$] at 30 μmol/kg ($p<0.001$) (Fig. 3).

ANOVA revealed a statistically significant difference in the total distance travelled during the 120 min conditioning session for the animals treated with the different doses of morphine [$F(5, 68)=90.71$, $p<0.001$]. Post-hoc comparison indicated that the locomotor activity was significantly increased ($p<0.05$) for the animals treated with 20, 30 and 50 μmol/kg doses of morphine when compared to the saline group. Increased locomotor activity was also recorded after treatment with M6G [$F(5, 65)=54.17$, $p<0.001$]. Post hoc comparison revealed statistically significantly increased ($p<0.05$) locomotor activity after treatment with M6G 30 and 50 μmol/kg compared to the saline group. M3G did not alter locomotor activity compared to saline (data not shown).

One mouse developed seizures in the lower limbs a few minutes following injection with M3G 240 μmol/kg on conditioning day 2 and was immediately sacrificed. The preceding injection was uncomplicated and no macroscopic damages were seen to internal organs at post mortem autopsy.

4. Discussion

This study revealed that morphine and M6G caused CPP, whereas M3G caused a tendency to CPA, compared to saline treated mice. Using a biased paradigm, the magnitude of the CPP induced by morphine and M6G varied depending on which compartment the animals were conditioned in. The saline treated mice showed a preference to the "closed" compartment.

It is well known that morphine has rewarding effects (Tzschentke, 1998), but this is not well studied for M6G. We are only aware of one previous study where CPP was induced after treatment with M6G in rats (Abbott and Franklin, 1991). S.c. morphine and M6G were approximately equipotent, but M6G was 146 times more potent than morphine when administered intracerebroventricularly to rats. Abbott administered only 2 different doses of M6G (0.25 and 2.0 mg/kg) s.c., and only the highest dose induced CPP. An equimolar dose of morphine was not tested. Lower doses of morphine induced CPP. In another study intranigral injection of M6G did not induce CPP (Baumeister et al., 1993). This might have been due to a dose–response effect, where the chosen doses were too low or to high, rather than the fact that intranigral M6G is unable to induce CPP. The antinociceptive effect of M6G has been investigated in different animal species after both systemic and central administration. Compared to morphine the potency ratios are reported from 1.6 to 4 following systemic administration and 13 to 800 following central administration (Christrup, 1997). Morphine and M6G are competitive agonists for the μ-opioid receptor. M6G has a slightly lower affinity for the receptor and slightly higher efficacy than morphine (Kilpatrick and Smith, 2005). The lower potency of M6G after systemic administration might be due to less penetration across the blood–brain barrier (BBB). M6G is less lipophilic, but migrate trough the BBB as molecular

chameleons hiding their hydrophilic groups (Carrupt et al., 1991). A number of studies have addressed the different potency of morphine and M6G after central administration, and binding to different subgroups of receptors has been suggested (Pan et al., 2005). The molecular mechanisms are however still unknown, and further studies need to be done.

The 120 min conditioning sessions used in this study are very long, compared to other CPP studies. In pilot studies, we did not obtain CPP after treatment with M6G when shorter conditioning sessions were tested. Locomotor activity data revealed that increased activity after M6G injection was delayed and lasted longer than for morphine, leading us to extend the duration of the conditioning sessions compared to the times reported in the literature. Only a limited number of studies have investigated M6G in the CPP paradigm and one reason might be the difficulties of inducing CPP with the most usual durations of the conditioning sessions.

The present study shows a continuous increase in CPP with increasing doses up to a certain level for the animals conditioned in the closed compartment with both morphine and M6G, and that further increase gave a reduction in CPP. Similarly inverted U-shaped dose–effect curves have been described for other drugs (Bardo et al., 1995). In other studies CPP and CPA have been induced after injection of the same drug, depending on the administered dose (Bardo et al., 1995). The inverted U-shaped dose–response curves for morphine and M6G in the present study might indicate that a further increase in doses could induce CPA.

Increased locomotor activity after repeated administration of the same dose of M6G (sensitisation) can be recorded at least as long as for morphine (Handal et al., in press). These findings together with the results from the present study indicate that M6G might have effects that could be related to development of addiction.

The conditioning model is sensitive to both reward and aversion (Bardo and Bevins, 2000). In the present study M3G showed a tendency to aversive properties, although not statistically significant, indicating that it can influence the effects of morphine. It has been suggested that M3G may be responsible for side effects like neuroexcitatory behaviours (myoclonus and allodynia) following administration of high systemic doses of morphine, used in cancer pain management (Smith, 2000). M3G has been shown to produce neuroexcitatory effects when administered directly into the lateral ventricle of the Sprague–Dawley rat brain (Bartlett et al., 1994). In the present experiment one mouse developed seizures in the lower limbs a few minutes after the second injection of 240 μmol/kg M3G. It is not clear whether this was an effect of M3G or damage to a local nerve by the injection. We have never observed this effect after injection of any drug or saline, and injections of higher doses of M3G have been given repeatedly by the same technique in this and other studies in our lab, without similar result. Seizures induced by M3G are supposed to be mediated by centrally mechanisms that require M3G to cross the BBB in sufficient quantities to exceed the neuroexitatory threshold (Labella et al., 1979; Smith, 2000). The short time from injection to focal seizures in the present study indicates that the mechanisms are not centrally mediated. We have not found literature regarding seizures due to local injection of M3G.

In biased models of CPP, the interpretation of the results may be difficult because the drug effects may depend on interaction with unconditioned motivational stimuli from the apparatus. Some studies have revealed that drugs may cause CPP only if conditioned to the non-preferred side (Schenk et al., 1985; Heinrichs and Martinez, 1986; Nomikos and Spyraki, 1988; Cervo et al., 1993; Schechter, 1995; Cunningham et al., 2003). It has been discussed whether this is a fear-reducing rather than a rewarding effect (Schenk et al., 1985; Cunningham et al., 2003) and morphine has been shown to have anxiolytic properties (Costall et al., 1989; Dockstader and van der Kooy, 2001; Shin et al., 2003). The phenomenon may also be explained as a measurement issue. If pairing an alleged rewarding drug with the initially non-preferred cue, it can be viewed as a manipulation providing greater opportunity to see a conditioning effect, maximizing the potential shift in place preference (Cunningham et al., 2003). Mice tend to prefer dark rooms compared to open, and in the present study the saline treated mice showed a statistically significant preference for the "closed" compartment, compared to the "open". Maximum CPP was seen for different doses of morphine and M6G, depending on which compartment the animals were conditioned to. Also, a clear tendency to a significant effect of the conditioning compartment and/or an interaction with the dose used was seen (the lack of significance could be consequence of the low statistical power due to the small number of animals, seven, per cell). This could indicate that different drug effects, like rewarding and anxiolytic effects will appear at different concentrations of the drugs, depending on the contextual contingency.

The present study showed a dose response effect of locomotor activity after treatment with morphine and M6G, which was different from the dose–response seen for CPP on the test day. The locomotor activity after drug treatment on the three conditioning sessions did not differ significant, and only the results from the 3rd conditioning day are presented. Both CPP and locomotor activity are associated with increased levels of dopamine in the nucleus accumbens, but there appears to be very little, if any correlation, between locomotor activity and the potential of a drug to induce CPP (Tzschentke, 1998; Parkinson et al., 1999; Cadoni and Di Chiara, 2000; Spielewoy et al., 2000; Dockstader and van der Kooy, 2001; Murphy et al., 2001; Robinson et al., 2005; Hnasko et al., 2005), also in accordance with our findings. M3G did not alter locomotor activity compared to saline, and this is in concordance with a previous study by Handal et al. (2002).

5. Conclusion

In the present study we have shown that M6G induce CPP and may have rewarding effects, like morphine, regarding the contextual contingency. We also found that M3G induced a tendency for CPA. These results indicate that M6G may play an important role in reward, while the role of M3G is uncertain and needs to be further explored.

References

Abbott FV, Franklin KB. Morphine-6-glucuronide contributes to rewarding effects of opiates. Life Sci 1991;48:1157–63.

Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology (Berl) 2000;153:31–43.

Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. Neurosci Biobehav Rev 1995;19:39–51.

Bartlett SE, Cramond T, Smith MT. The excitatory effects of morphine-3-glucuronide are attenuated by LY274614, a competitive NMDA receptor antagonist, and by midazolam, an agonist at the benzodiazepine site on the GABAA receptor complex. Life Sci 1994;54:687–94.

Baumeister AA, Hurry M, Curtis W, Chaney TM, Wolf E, Leoni RR. The antinociceptive and motivational effects of intranigral injection of opioid agonists. Neuropharmacology 1993;32:1299–303.

Cadoni C, Di Chiara G. Differential changes in accumbens shell and core dopamine in behavioral sensitization to nicotine. Eur J Pharmacol 2000;387: R23–5.

Carrupt PA, Testa B, Bechalany A, el Tayar N, Descas P, Perrissoud D. Morphine 6-glucuronide and morphine 3-glucuronide as molecular chameleons with unexpected lipophilicity. J Med Chem 1991;34:1272–5.

Cervo L, Rossi C, Samanin R. Clonidine-induced place preference is mediated by alpha 2-adrenoceptors outside the locus coeruleus. Eur J Pharmacol 1993;238:201–7.

Christrup LL. Morphine metabolites. Acta Anaesthesiol Scand 1997;41:116–22.

Costall B, Jones BJ, Kelly ME, Naylor RJ, Tomkins DM. Exploration of mice in a black and white test box: validation as a model of anxiety. Pharmacol Biochem Behav 1989;32:777–85.

Cunningham CL, Ferree NK, Howard MA. Apparatus bias and place conditioning with ethanol in mice. Psychopharmacology (Berl) 2003;170:409–22.

Dambisya YM, Chan K, Wong CL. Further metabolic studies of codeine and morphine in mice pretreated with sympathomimetics. Methods Find Exp Clin Pharmacol 1992;14:773–80.

Dockstader CL, van der Kooy D. Mouse strain differences in opiate reward learning are explained by differences in anxiety, not reward or learning. J Neurosci 2001;21:9077–81.

Gong QL, Hedner J, Bjorkman R, Hedner T. Morphine-3-glucuronide may functionally antagonize morphine-6-glucuronide induced antinociception and ventilatory depression in the rat. Pain 1992;48:249–55.

Handal M, Grung M, Skurtveit S, Ripel A, Morland J. Pharmacokinetic differences of morphine and morphine-glucuronides are reflected in locomotor activity. Pharmacol Biochem Behav 2002;73:883–92.

Handal M, Ripel A, Aasmundstad TA, Skurtveit S, Morland J. Morphine-3-glucuronide inhibits morphine induced, but enhances morphine-6-glucuronide induced locomotor activity in mice. Pharmacol Biochem Behav, in press.

Heinrichs SC, Martinez Jr JL. Modification of place preference conditioning in mice by systemically administered [Leu]enkephalin. Behav Brain Res 1986;22:249–55.

Hnasko TS, Sotak BN, Palmiter RD. Morphine reward in dopamine-deficient mice. Nature 2005;438:854–7.

Kilpatrick GJ, Smith TW. Morphine-6-glucuronide: actions and mechanisms. Med Res Rev 2005;25:521–44.

Klepstad P, Kaasa S, Borchgrevink PC. Start of oral morphine to cancer patients: effective serum morphine concentrations and contribution from morphine-6-glucuronide to the analgesia produced by morphine. Eur J Clin Pharmacol 2000;55:713–9.

Kuo CK, Hanioka N, Hoshikawa Y, Oguri K, Yoshimura H. Species difference of site-selective glucuronidation of morphine. J Pharmacobiodyn 1991;14: 187–93.

Labella FS, Pinsky C, Havlicek V. Morphine derivatives with diminished opiate receptor potency show enhanced central excitatory activity. Brain Res 1979;174:263–71.

Lipkowski AW, Carr DB, Langlade A, Osgood PF, Szyfelbein SK. Morphine-3-glucuronide: silent regulator of morphine actions. Life Sci 1994;55:149–54.

Murphy NP, Lam HA, Maidment NT. A comparison of morphine-induced locomotor activity and mesolimbic dopamine release in C57BL6, 129Sv and DBA2 mice. J Neurochem 2001;79:626–35.

Nomikos GG, Spyraki C. Cocaine-induced place conditioning: importance of route of administration and other procedural variables. Psychopharmacology (Berl) 1988;94:119–25.

Pan L, Xu J, Yu R, Xu MM, Pan YX, Pasternak GW. Identification and characterization of six new alternatively spliced variants of the human mu opioid receptor gene, Oprm. Neuroscience 2005;133:209–20.

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ. Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. J Neurosci 1999;19:2401–11.

Robinson S, Sandstrom SM, Denenberg VH, Palmiter RD. Distinguishing whether dopamine regulates liking, wanting, and/or learning about rewards. Behav Neurosci 2005;119:5-15.

Schechter MD. Cocaethylene produces conditioned place preference in rats. Pharmacol Biochem Behav 1995;51:549–52.

Schenk S, Ellison F, Hunt T, Amit Z. An examination of heroin conditioning in preferred and nonpreferred environments and in differentially housed mature and immature rats. Pharmacol Biochem Behav 1985;22:215–20.

Shin IC, Kim HC, Swanson J, Hong JT, Oh KW. Anxiolytic effects of acute morphine can be modulated by nitric oxide systems. Pharmacology 2003;68:183–9.

Smith MT. Neuroexcitatory effects of morphine and hydromorphone: evidence implicating the 3-glucuronide metabolites. Clin Exp Pharmacol Physiol 2000;27:524–8.

Spielewoy C, Gonon F, Roubert C, Fauchey V, Jaber M, Caron MG, et al. Increased rewarding properties of morphine in dopamine-transporter knockout mice. Eur J Neurosci 2000;12:1827–37.

Suzuki N, Kalso E, Rosenberg PH. Intrathecal morphine-3-glucuronide does not antagonize spinal antinociception by morphine or morphine-6-glucuronide in rats. Eur J Pharmacol 1993;249:247–50.

Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol 1998;56:613–72.