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Comparison of the antinociceptive effects of morphine, methadone, buprenorphine and codeine in two substrains of Sprague–Dawley rats

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Abstract

Sprague–Dawley rats from two different vendors, Möllegård, Denmark and B&K Universal, Sweden, have been tested for the antinociceptive effect of morphine, methadone, buprenorphine and codeine on the hot plate. Morphine and methadone had significantly weaker effect in Möllegård rats compare to B&K rats. In contrast, the effect of buprenorphine was stronger in Möllegård rats than in B&K rats and the effect of codeine was similar in the two substrains. Plasma levels of morphine, morphine-6-glucuronide, morphine-3-glucuronide, buprenorphine and norbuprenorphine were determined at two time points after drug injection. Möllegård rats had significantly lower mean plasma level of morphine and significantly higher ratio of morphine-3-glucuronide/morphine at 30 min, compared to B&K rats. No difference was seen for the metabolism of buprenorphine in the two substrains. The results suggest that Möllegård rats metabolize morphine to morphine-3-glucuronide to a greater extent than B&K rats, and this may at least partly underlie the substrain difference in the effect of morphine. It is also suggested that the antinociceptive mechanisms of buprenorphne may be different from those of morphine and methadone.

Keywords: Genetic difference; Opioid; Pharmacokinetic; Pharmacodynamic

1. Introduction

Perception of pain and sensitivity to analgesics are highly variable both among human individuals (Coghill et al., 2003) and rodent strains (Devor and Raber, 1990; Inbal et al., 1980; Mogil et al., 1996b; Wiesenfeld and Hallin, 1981). In humans opiate analgesics display large clinical and experimental variability in their efficacies, side effects and tolerance liability (Chapman et al., 1990; Galer et al., 1992; Isselee et al., 1997). Important qualitative and quantitative differences in pain and analgesia have also been reported in animal studies (see Mogil, 1999 for review). Significant genetic differences in the endogenous opioid system and in the response to noxious stimuli are known to be present in rats (Sitsen and de Jong, 1983). The antinociceptive effect of morphine and other opioids as well as the rate of tolerance development vary among rat and mice strains, indicating an important role of genetic factors (Elmer et al., 1998; Hoffmann et al., 1998; Kest et al., 2002; Mas et al., 2000; Mogil et al., 1996a; Plesan et al., 1999; Vaccarino and Couret, 1995). Genetic polymorphism of relevance to analgesia has been documented for the neuronal cytochrome P450 necessary for transformation of codeine to morphine: cytochrome P2D6 in humans and its equivalent in rats: cytochrome P2D1 (Chen et al., 1988; Cleary et al., 1994; Poulsen et al., 1996).

Intrastrain variability in basal nociceptive sensitivity and in the development of neuropathic behaviors has been previously described in our laboratory (Xu et al., 2001). Differences in the development of neuropathic pain have been also noted between two substrains of Sprague–Dawley rats delivered from two different vendors: B&K Universal, Sweden, and Möllegård, Denmark, with Möllegård rats being more prone to neuropathic pain. We have

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previously observed that Möllegård rats also exhibited reduced response to the antinociceptive effect of morphine (Bulka et al., 2002). This has raised an interesting possibility that there may be a correlation between the development of neuropathic pain and response to opioids. In the present study, we tested the antinociceptive effects of four different opioids, morphine, methadone, codeine and buprenorphine, in these two substrains of SD rats in order to further study the relationship between sensitivity to opioids and the development of neuropathic pain. We have also examined the metabolism of morphine and buprenorphine in these two substrains. This is important because a pharmacokinetic or pharmacodynamic mechanism for the differential sensitivity to exogenous administered drugs may not reflect sensitivity to endogenous opioid peptides.

All studied opioids have preference for the μ-opioid receptor, but differ in their pharmacokinetics, pharmacodynamics and pharmacology (Gourlay, 1999). Morphine shows the greatest relative preference for the μ-opioid receptor, has active metabolites, and is the active metabolite of codeine (Chen et al., 1988). Methadone and buprenorphine show significant binding to δ-opioid receptors at two to four times greater concentration than their μ-receptor binding affinities (Gourlay, 1999). Furthermore, the D- and L-isomers of methadone act also as *N*-methyl-D-aspartate (NMDA) receptor antagonists (Ebert et al., 1995; Gorman et al., 1997). Some mechanisms, which mediate buprenorphine-induced antinociception, differ from those of classical μ-opioid receptor agonists (McCormack, 1999). Compared to morphine, buprenorphine produced stronger analgesia in the cold ethanol tail-flick test and was more potent in alleviating neuropathic behaviors in SD rats (Kouya et al., 2002; Wang et al., 1995). Unlike morphine, buprenorphine analgesia in rats is not blocked by intrathecal pretreatment with pertussis toxin and is uniquely sensitive to the effects of K_{ATP} channel openers and blockers (Ocana et al., 1995; Wheeler-Aceto and Cowan, 1991).

2. Materials and methods

Adult male Sprague–Dawley rats from two different vendors, Möllegård, Denmark and B&K Universal, Sweden, weighing 250–300 g, were used. The animals were housed 4/cage with food and water available ad libidum in a 12:12 h light/dark cycle. The experiments were carried out according to the Ethical Guidelines of the International Association for the Study of Pain and were approved by the local animal research ethics committee. Antinociception was assessed with a hot plate (IITC, Woodland Hills, CA) maintained at 54 ± 0.1 °C. The latency to licking a hind paw was measured with an accuracy of 0.1 s and the cutoff time was 30 s to avoid tissue damage. The rats were trained on the hot plate for 4–5 days in order to obtain a stable baseline response, and on the day of the experiment two control latencies were averaged before drug administration.

All rats were injected subcutaneously (s.c.) with an opiod (codeine, morphine, methadone or buprenorphine) or saline. The doses were as follows: 2, 6 or 10 mg/kg morphine, 1, 3 or 5 mg/kg methadone, 0.03, 0.1 or 0.3 mg/kg buprenorphine, 10, 30 or 100 mg/kg codeine, and were chosen according to previous studies in our laboratory on their dose-dependent antinociceptive effect in the hot plate test (Hoffmann et al., 1998; Kouya et al., 2002; Plesan et al., 1999). Each animal was injected only once. Response latency was measured at 15 (only buprenorphine), 30, 60, 90, 120, 180 and 240 min after drug administration. All experiments were performed blindly so the experimenter was not aware of the drug and the dose, but knew which rat substrain was tested.

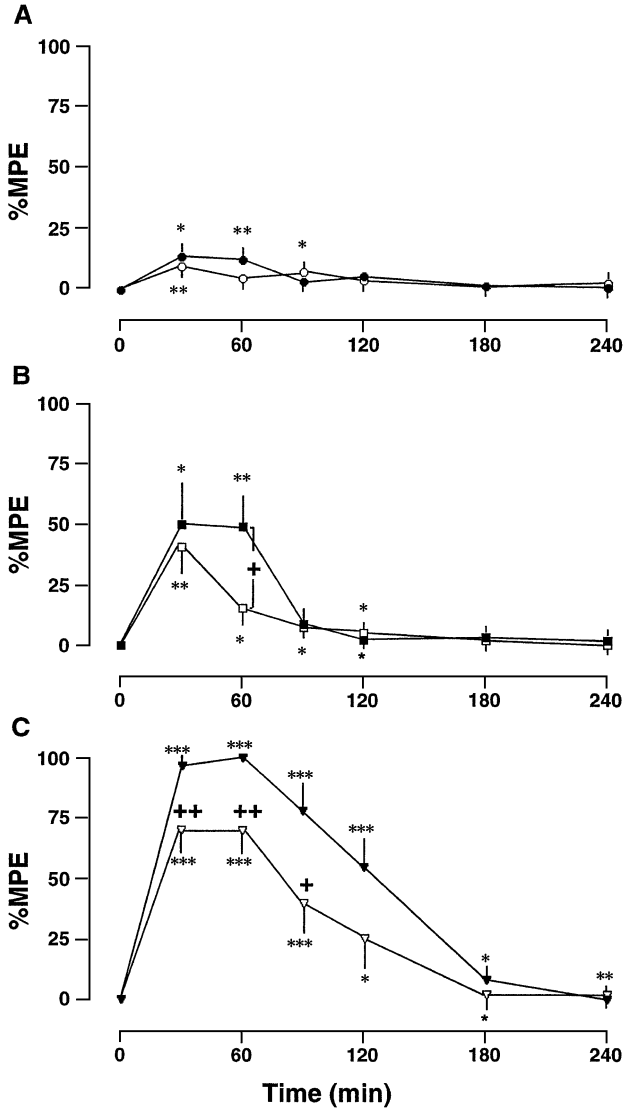

Fig. 1. Effects of systemic morphine at (A) 2, (B) 6 and (C) 10 mg/kg on the lick latency in B&K (filled symbols) and Möllegård (open symbols) rats. The number of animals in each group varied between 8 and 20. The data are expressed as mean ± S.E.M. Two-way ANOVA indicated significant difference between the Möllegård and B&K and $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to pre-drug baseline value (time 0) with paired *t*-test. $^{+}P<0.05$, $^{++}P<0.01$, compared between two groups with unpaired *t*-test.

Plasma levels of morphine and buprenorphine and their metabolites were measured in a separate experiment. The antinociceptive effect of morphine (10 mg/kg) was tested 30 and 120 min after administration and for buprenorphine (0.3 mg/kg) at 15 and 60 min. After the second test the animals were immediately decapitated. The blood samples ($N=6$ in each group) were centrifuged and stored at -20 °C until analysis. Plasma concentrations of morphine and its metabolites morphine-3-glucoronide and morphine-6-glucoronide and buprenorphine and its metabolite norbuprenorphine were measured by liquid chromatography with mass spectrometry detection.

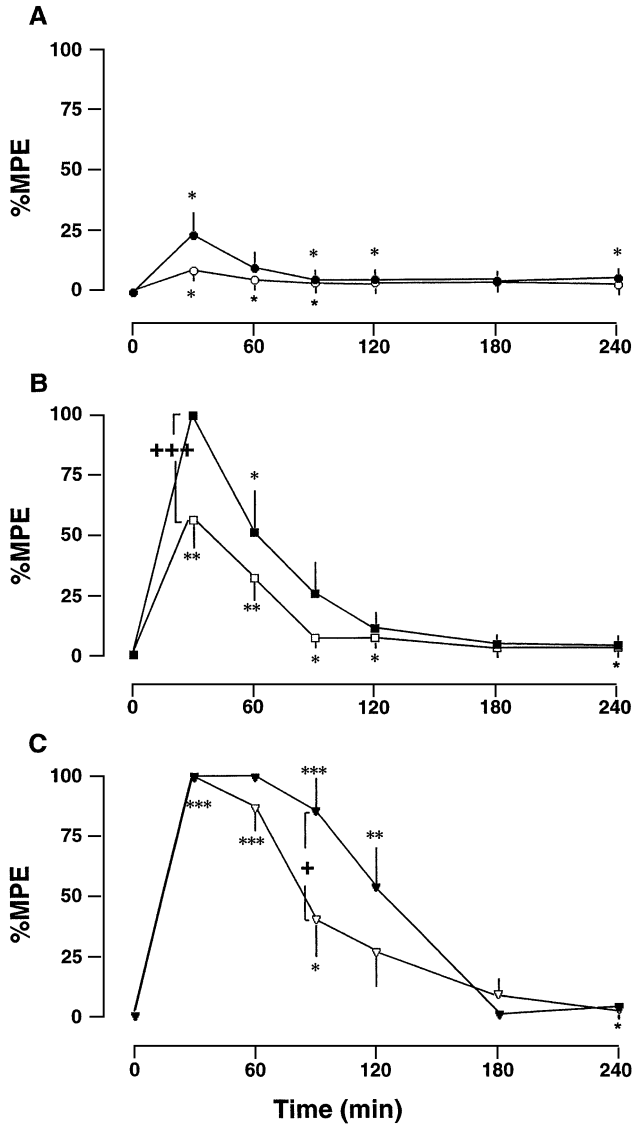

Fig. 2. Effects of systemic methadone at (A) 1, (B) 3, and (C) 5 mg/kg on the lick latency in B&K (filled symbols) and Möllegård (open symbols) rats. $N=8$ in each group. The data are expressed as mean ± S.E.M. Two-way ANOVA indicated significant difference between the Möllegård and B&K rats and $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to baseline value with paired t-test. $^{+}P<0.05$, $^{+++}P<0.001$ compared between two groups with unpaired t-test.

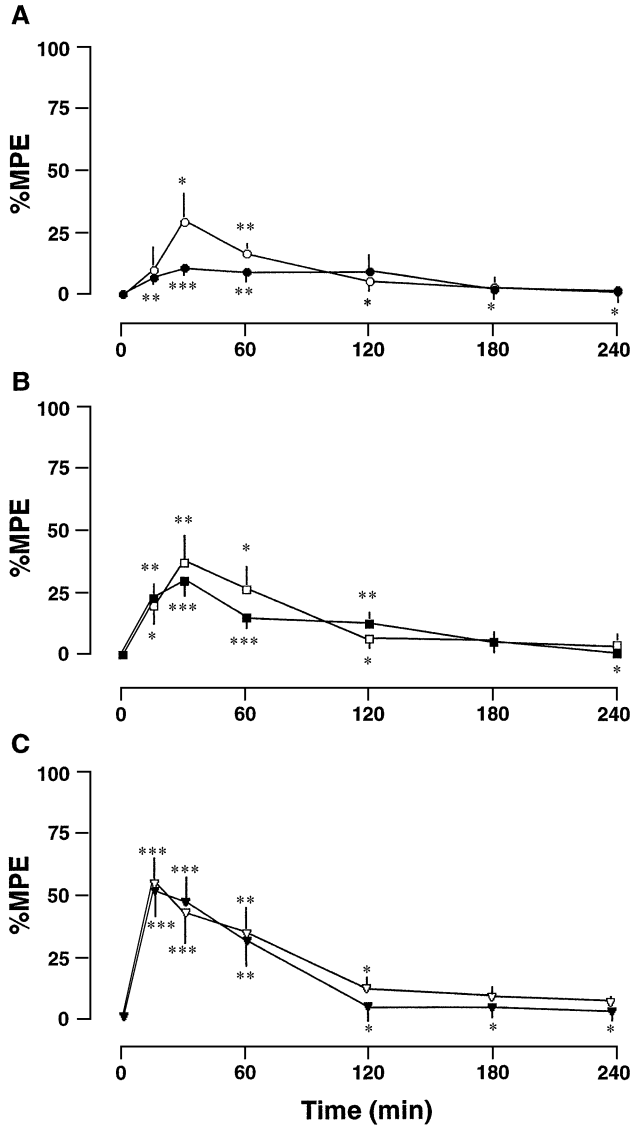

Fig. 3. Effects of systemic buprenorphine at (A) 0.03, (B) 0.1 and (C) 0.3 mg/kg on the lick latency in B&K (filled symbols) and Möllegård (open symbols) rats. The number of animals varied between 8 and 22 in each group. The data are expressed as mean ± S.E.M. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to baseline value with paired t-test.

The drugs were dissolved in 0.9% physiological saline and injected in a volume of 1 ml/kg. The response latencies for the highest doses of morphine and buprenorphine obtained in the hot plate test in the behavioral and pharmacokinetic parts of the study have been combined. The pre-drug baseline latency was compared in the two groups using unpaired t-test. All other drug data are presented as maximum possible effect (%MPE) calculated according to the formula:

$$\%\mathrm{MPE} = \frac{(\text{post} - \text{drug latency}) - (\text{pre} - \text{drug latency})}{30 - (\text{pre} - \text{drug latency})} \times 100$$

The behavioral data are presented as mean ± S.E.M. The data were analyzed with analysis of variance (ANOVA) with repeated measures followed by the paired *t*-test. The unpaired *t*-test was used for comparisons across groups. The ED 50% was calculated from the regression plot of mean of %MPEs for the three doses of each drug. Plasma concentrations are presented as mean ± S.E.M. Plasma concentrations and plasma ratios between the two substrains were compared by unpaired *t*-test.

3. Results

There was a significant difference in mean pre-drug baseline response latency between the Möllegård rats (5.11 s, $n=119$) and B&K rats (4.51 s, $n=148$) rats ($P<0.001$). Therefore, all data for drug effects are expressed as %MPE.

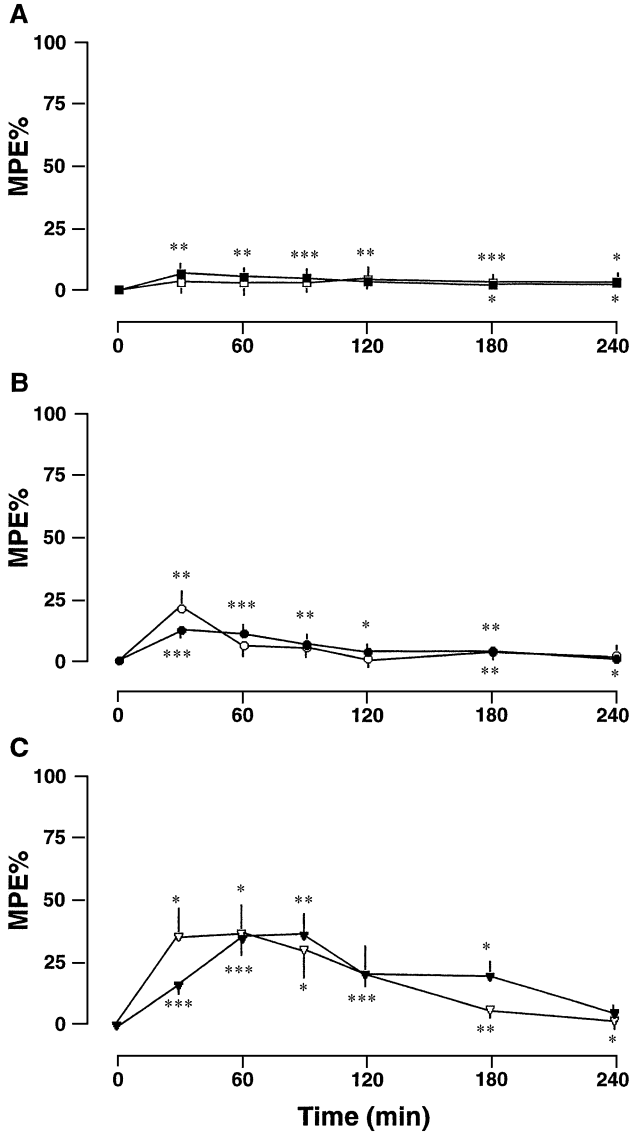

Fig. 4. Effects of systemic codeine at (A) 10, (B) 30 and (C) 100 mg/kg on the lick latency in B&K (filled symbols) and Möllegård (open symbols) rats. The number of animals varied between 7 and 18 in each group. The data are expressed as mean ± S.E.M. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ compared to baseline value with paired *t*-test.

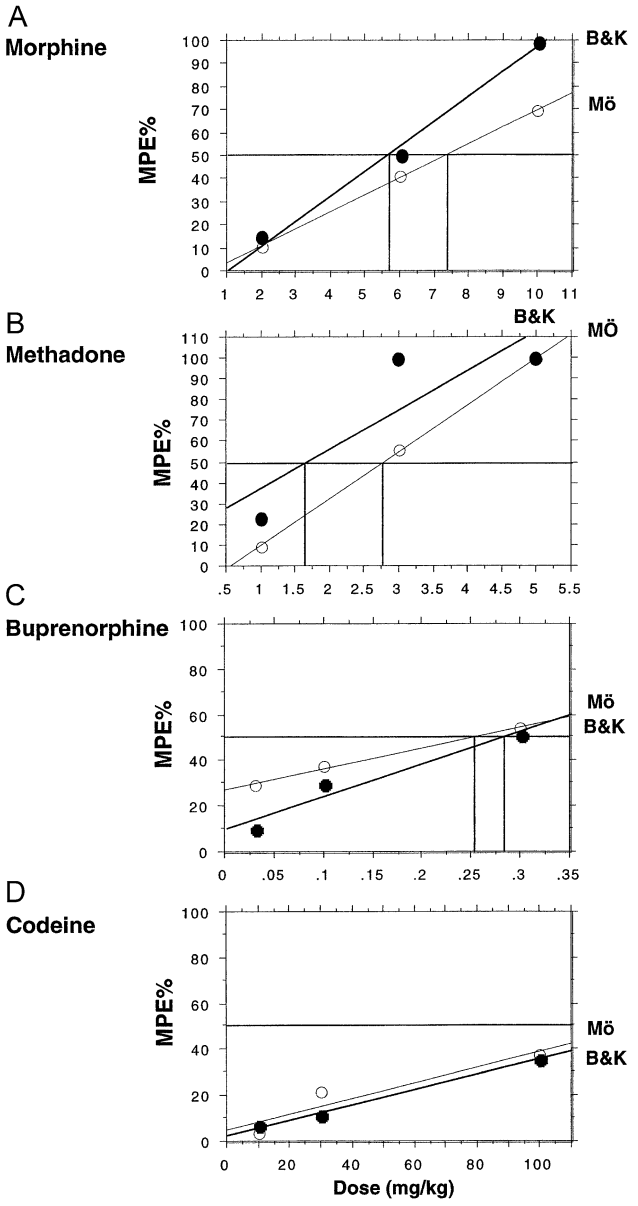

Fig. 5. The regression plot of the effect of (A) morphine, (B) methadone, (C) buprenorphine and (D) codeine at the three tested doses, presented as %MPE in the B&K rats (filled symbols) and Möllegård rats (open symbols).

3.1. Effects of morphine

Morphine produced dose-dependent antinociception at three doses in both substrains ($F2,42=55.9$, $P<0.001$, Fig. 1). ANOVA with repeated measures also revealed an overall stronger effect in B&K rats than in Möllegård rats ($F1,42=22.9$, $P<0.001$). A similar weak antinociception was observed following 2 mg/kg morphine in both substrains (Fig. 1A). At 6 mg/kg, morphine had similar max-

imum effect in both Möllegård rats and B&K rats (41.3% and 50% MPE, respectively), but the antinociception at 60 min was significantly stronger in B&K rats than in Möllegård rats (Fig. 1B). At 10 mg/kg, morphine was significantly more potent in B&K rats than in Möllegård rats at 30, 60 and 90 min after administration (Fig. 1C). All B&K rats had 100% MPE at 60 min whereas the highest MPE for Möllegård rats was 68%. The ED50 was 5.6 mg/kg for B&K rats (95% CL, 2.6 to 11.2 mg/kg) and 8.6 mg/kg for Möllegård rats (95% CL, 2.9 to 30.6 mg/kg) (Fig. 5).

3.2. Effects of methadone

Methadone produced dose-dependent antinociception in both substrains ($F2,42 = 73$, $P < 0.001$) and the effect was significantly weaker in the Möllegård rats than in the B&K rats ($F1,42 = 11.8$, $P < 0.01$). At 1 mg/kg, methadone in both substrains produced weak antinociception (Fig. 2A). At 3 mg/kg, methadone had a significantly stronger effect in B&K rats than Möllegård rats. At this dose, all B&K rats reached the cutoff value, whereas only two of eight of Möllegård rats did so. The highest dose of methadone, 5 mg/kg, produced a maximal effect in both substrains at 30 min. The antinociception was stronger at 90 min in B&K rats. The ED50 was 5.6 mg/kg for B&K rats (95% CL, 2.6 to 11.2 mg/kg) and 8.6 mg/kg for Möllegård rats (95% CL, 2.9 to 30.6 mg/kg) (Fig. 5).

3.3. Effects of buprenorphine

Buprenorphine at 0.03, 0.1 and 0.3 mg/kg produced dose-dependent, significant antinociception ($F2,48 = 6.6$, $P < 0.01$) and the effect was marginally, but significantly, stronger in Möllegård rats than in the B&K rats ($F1,48 = 5.6$, $P < 0.05$) (Fig. 3). The mean %MPE for Möllegård rats at the highest dose was 54.1 and for B&K rats was 51.6. The regression for Möllegård rats is not significant. The ED50 for B&K rats was 0.52 mg/kg (0.20–5.02 mg/kg, 95% CL) (Fig. 5).

3.4. Effects of codeine

Codeine at 10, 30 and 100 mg/kg had a weak, dose-dependent, antinociceptive effect ($F2,61 = 28.4$, $P < 0.001$) and there was no significant difference between the two strains ($F1,61 = 0.1$, $P > 0.05$) (Fig. 4). The %MPE reached by the Möllegård rats at the highest dose was 37.7 and for the B&K rats 35.4.

3.5. Morphine and buprenorphine pharmacokinetics in Möllegård and B&K rats

Thirty minutes after administration of 10 mg/kg morphine, B&K rats had a mean plasma level of morphine of 5035 nmol/l and a mean level of morphine-3-glucoronide of 1999 nmol/l (Fig. 6). Corresponding levels in the Möllegård rats were 2751 nmol/l for morphine and 2863 nmol/l for the metabolite. The mean ratio of morphine-3-glucoronide/morphine at 30 min post dosing was 0.43 (range 0.19–0.71) for the B&K rats and 1.1 (range 0.78–1.8) for the Möllegård rats ($P < 0.01$). Two hours after dosing, morphine levels were equally low in the two substrains (396 nmol in Möllegård rats and 454 nmol in B&K rats), while the Möllegård rats still tended to have a higher morphine-3-glucoronide/morphine ratio of 5.1, compared with the B&K rats with a ratio of 2.9. Levels of the active metabolite morphine-6-glucoronide were, even though significantly higher in the B&K rats, very low in both substrains.

Mean plasma levels of buprenorphine and norbuprenorphine did not differ between the two substrains of rats at

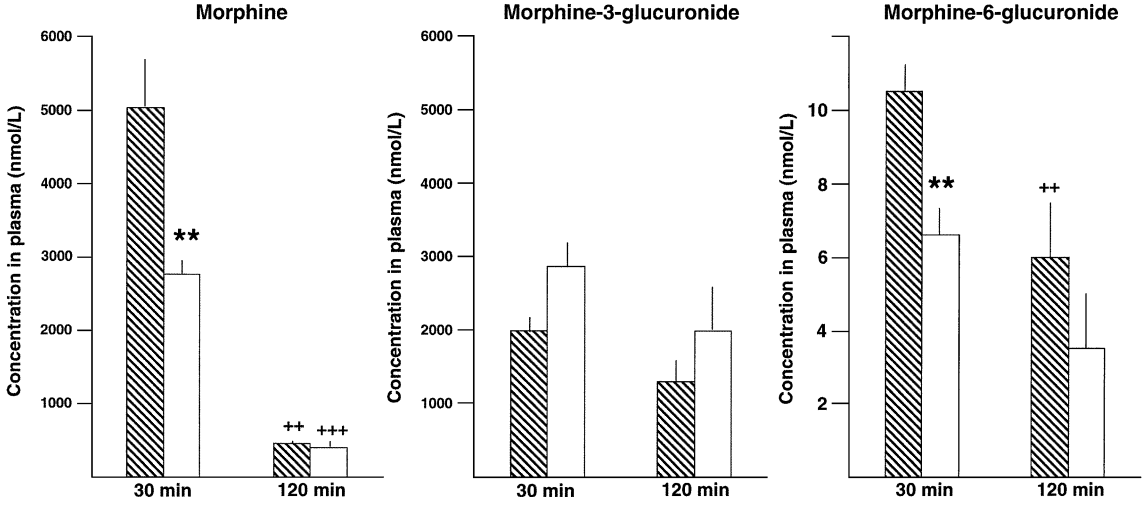

Fig. 6. Plasma concentrations of morphine, morphine-3-glucoronide, and morphine-6-glucoronide at 30 and 120 min post dosing s.c. 10 mg/kg morphine in Möllegård (open bars) and B&K (hatched bars) rats. The data are expressed as mean ± S.E.M. $^{**}P < 0.01$ compared between two substrains with unpaired *t*-test. $^{++}P < 0.01$, $^{+++}P < 0.001$ compared between two time points with paired *t*-test.

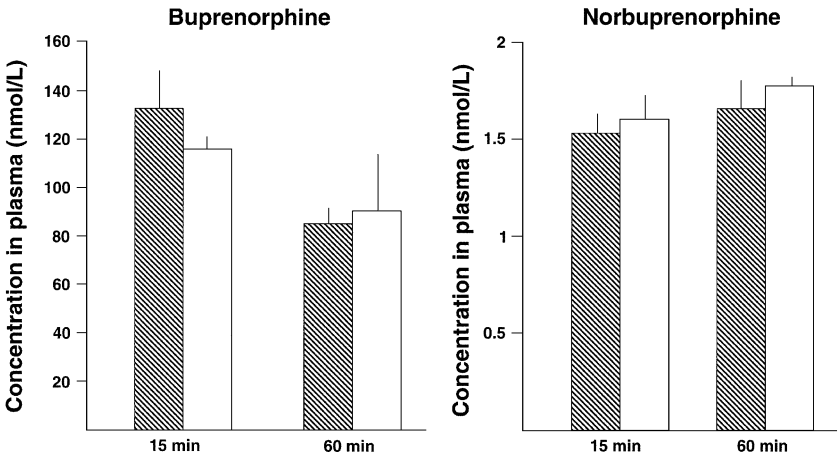

Fig. 7. Plasma concentrations of buprenorphine and norbuprenorphine at 15 and 60 min post dosing s.c. 0.3 mg/kg buprenorphine in Möllegård (open bars) and B&K (hatched bars) rats. The data are expressed as mean ± S.E.M.

either 15 or 60 min after administration of 0.3 mg/kg buprenorphine (Fig. 7).

4. Discussion

The observed differences in these two substrains in plasma levels of morphine and its metabolites correspond to the analgesic effects. Möllegård rats had significantly lower mean plasma level of morphine and significantly higher ratio of morphine-3-glucoronide/morphine at 30 min, with tendency to higher ratio at 120 min, compared to B&K rats. The morphine metabolite morphine-3-glucoronide has an excitatory effect which reduces analgesia (Osborne et al., 1992) and the higher ratio in Möllegård rats can thus underlie their weaker response to morphine compared to B&K rats. Levels of the potent analgesic active metabolite morphine-6-glucoronide were very low in both substrains and would not have contributed to the analgesic effect (Gong et al., 1992). We conclude, therefore, that the Möllegård rats metabolize morphine to morphine-3-glucoronide to a greater extent compared with the B&K substrain, and that this difference in the pharmacokinetic profiles between the two substrains could partially explain the difference in analgesic effects. However, in contrast to the present results, no kinetic basis for difference in morphine sensitivity has been found between Sprague–Dawley–U rats and Wistar rats (Mas et al., 2000).

Although less prominent, methadone also had reduced antinociceptive effect in Möllegård rats than in B&K rats. In contrast to morphine, methadone has no active metabolite. Thus, a reduced effect for methadone may be due to more rapid elimination or additional mechanisms unrelated to pharmacodynamic or pharmacokinetic reasons. We have previously found that administration the NMDA receptor antagonist dextrometorphan produced more profound potentiation of the antinociceptive effect of morphine in Möllegård rats than in the B&K rats. Opioid administration is known to activate NMDA receptors for glutamate, which reduces opioid efficacy and, conversely, coadministration of NMDA receptor antagonists with opioids potentiates antinociception (see Wiesenfeld-Hallin, 1998 for review). Thus, it is also possible that Möllegård rats may have higher absolute number and/or density of NMDA receptors, which antagonizes opioid effect.

From a pharmacological point of view, the level of antinociception produced by any opioid is also dependent on the intrinsic efficacy of the drug (Morgan et al., 1999b; O'Callaghan and Holtzman, 1975). Profound differences in sensitivity to different opioids between several strains of rat were most noted with the use of low efficacy opioids (Morgan et al., 1999a). Morphine has higher intrinsic efficacy than buprenorphine and codeine (Paronis and Holtzman, 1992) but no strain difference was seen for codeine and buprenorphine has in fact stronger effect in the Möllegård rats. This would suggest that pharmacological mechanisms such as differences in density of opioid receptor and the intracellular mechanisms determining μ-opioid efficacy (e.g., the degree and/or the amount of G protein activation) (Elmer et al., 1995; Sudakov et al., 1993; Morgan et al., 1999a; Selley et al., 1997) did not play a role in observed strain difference for morphine. These results would also support the notion that the antinociceptive mechanisms for buprenorphine are different from that of classical μ-opioids (McCormack, 1999) since they exhibited opposite substrain sensitivity.

There is extensive evidence for significant genetic influence on morphine potency within and across nociception assays (Elmer et al., 1998; Kest et al., 2002; Mas et al., 2000; Mogil et al., 1996a). Here we present variability between rats from different vendors, but of the same strain. The differences observed in the present study may be due to genetic and/or environmental factors. Since the environmental conditions of the experiments are held constant, the genetic factors seem to influence differences in morphine potency. However, the two substrains of SD rats were

delivered from two vendors, thus the observed sensitivity to morphine may have been affected by uncontrolled environmental factors, fixed early in life (Shir et al., 1997).

Acknowledgements

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