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The role of descending fibers from the rostral ventromedial medulla in opioid analgesia in rats

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Abstract

There has been controversy as to whether the contribution of descending fibers from the rostral ventromedial medulla to opioid analgesia depends on the nature of the noxious stimulus eliciting pain. In the present study, inactivation of descending fibers by microinjection of muscimol (50 ng) in the rostral ventromedial medulla abolished morphine analgesia in the tail immersion and hot plate tests but decreased morphine analgesia by 60% in the formalin test. Analysis of the dose–response relation for morphine after inactivation of descending fibers revealed that, except for the tail immersion test, high doses of morphine could not overcome the block induced by muscimol. Also, morphine analgesia elicited supraspinally was not detectable when descending fibers were inactivated, suggesting that the analgesic effect of morphine in the brain requires a relay via the rostral ventromedial medulla. The analgesic effect of buprenorphine also depends on the integrity of descending fibers from the rostral ventromedial medulla. The results indicate that descending fibers from the rostral ventromedial medulla are critically important to the analgesic effect of opioids, regardless of the type of noxious stimulation eliciting pain. Residual analgesic effects of opioids after inactivation of descending fibers may be due to peripheral effects in the presence of inflammation.

Keywords: Formalin test; Hot plate; Tail immersion; Morphine; Nociception; Buprenorphine

1. Introduction

Fibers which descend from the rostral ventromedial medulla via the dorsolateral funiculus to the dorsal horn of the spinal cord are important mediators of the analgesic effect of morphine. However, there has been controversy as to whether the role of this bulbo-spinal pathway depends on the nature of the noxious stimulus eliciting pain. For instance, electrolytic lesions of the rostral ventromedial medulla or bilateral lesions of the dorsolateral funiculus markedly decreased the analgesic effect of systemic morphine in the tail flick test, but not in the formalin test (Abbott and Melzack, 1982; Abbott et al., 1982; Ryan et al., 1985). More recent studies found that lesions of the dorsolateral funiculus decreased the analgesic effect of systemic morphine in the formalin test (Abbott et al., 1996; Gogas et al., 1996) but, because lesions of the dorsolateral funiculus have been reported to increase nociception (Abbott et al., 1996), it is not clear whether fibers from the rostral ventromedial medulla are required for the expression of morphine analgesia, or whether they merely regulate sensitivity to pain.

Some studies have suggested that morphine analgesia in the formalin test involves specific forebrain areas. Morphine analgesia is abolished in rats transected rostral to the pons, suggesting that forebrain areas participate in the analgesic effect of morphine in the formalin test (Matthies and Franklin, 1992). More recent studies implicate the amygdala as a cortical site (Manning and Mayer, 1995a,b; Matthies and Franklin, 1995). Whether the action of morphine at these supraspinal sites depends on a relay via the rostral ventromedial medulla is not clear.

In the present paper, we compared the contribution of pathways descending from the rostral ventromedial medulla to the analgesic effect of morphine in the tail immersion, hot plate and formalin tests. The first experiment attempted to determine whether inactivation of descending fibers from

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the rostral ventromedial medulla similarly affects morphine analgesia in the three tests. The second experiment assessed the dose–response relation for morphine after inactivation of the rostral ventromedial medulla. The goal of the third experiment was to determine whether morphine analgesia induced supraspinally depends on a relay via the rostral ventromedial medulla. Finally, to expand the results obtained with morphine to another opioid, experiment 4 explored the contribution of descending fibers from the rostral ventromedial medulla to the analgesic effect of buprenorphine.

2. Materials and methods

The present experiments were reviewed by the Ethics Subcommittee of the University Animal Care Committee, McGill University, (Montreal, Quebec, Canada) and carried out according to guidelines of the Canadian Council on Animal Care.

2.1. Animals

Male Wistar albino rats (Charles River, St.-Constant, Quebec, Canada) weighing between 175 and 225 g were housed individually in a room maintained at 22 ± 0.5 °C with a 12-h light/dark cycle. Food (Purina Rat Chow) and water were freely available.

2.2. Inactivation of descending fibers from the rostral ventromedial medulla

Descending pathways were inactivated by microinjection of the $GABA_A$ receptor agonist muscimol in the rostral ventromedial medulla. Muscimol has been used previously to inactivate populations of neurons rich in GABA receptors (Majchrzak and Di Scala, 2000) such as the rostral ventromedial medulla (Gilbert and Franklin, 2001; Meng et al., 1998). Descending fibers from the rostral ventromedial medulla are under GABAergic control and the soma and dendrites of these spinally projecting neurons possess $GABA_A$ receptors (Cho and Basbaum, 1991). By increasing the permeability of the cell to chloride and, therefore, increasing the influx of chloride anions entering the cell, muscimol inhibits the firing of neurons projecting to the dorsal horn of the spinal cord (Vicini, 1999). At the behavioural level, microinjection of muscimol in the rostral ventromedial medulla produces effects similar to those observed after microinjection of the local anaesthetic lidocaine (Sandkühler et al., 1987) or lesioning of the area (Proudfit, 1981). Silencing of descending fibers by GABAergic agonists has also been confirmed electrophysiologically (Heinricher et al., 1991). The use of a reversible, specific pharmacological agent avoids some problems typically encountered with lesions. It restricts the functional lesion to particular classes of cells, eliminates damage to fibers of passage and bypasses compensatory mechanisms that may mask the effects of chronic lesions over time (Berge et al., 1983; Proudfit, 1981).

2.3. Surgery and histology

Animals were premedicated with 4 mg/kg xylazine i.m. and anaesthetised with 45 mg/kg sodium pentobarbitol i.p. A permanent indwelling stainless steel guide cannula (23 g) was stereotaxically implanted 2.2 mm dorsal to the nucleus raphe magnus. The coordinates were AP: −10 mm; LR: 0; DV: −8.3 mm to Bregma (Paxinos and Watson, 1998). For the experiment evaluating the effect of supraspinal morphine, an additional cannula was implanted in the lateral ventricle (i.c.v.) according to the following coordinates: AP: −0.9 mm; LR: ±1.5 mm; DV: −2.7 mm in relation to Bregma (Paxinos and Watson, 1998). Half of the cannulae were placed in the left ventricle and half in the right ventricle, alternately. The cannula was secured to the skull with stainless steel screws and dental acrylic. A protective stylet was inserted in order to avoid clogging of the guide cannula during the 1-week recovery period.

After completion of behavioural testing, Indian ink (Speedball India Ink, Statesville, NC) was microinjected through the guide cannula. Rats were overdosed with 30% chloral hydrate and perfused intracardiacally with 0.9% saline followed by 10% formaldehyde. Brains were removed and fixed for 6–10 days in a solution of 10% formalin and 30% sucrose. Brains were cut in the coronal plane and 30-μm sections were taken at 120-μm intervals, mounted on gelatinised glass slides and stained with Cresyl Violet (Cellpoint Scientific, Rockville, MD). Only animals with cannula tips within 0.5 mm of the nucleus raphe magnus at the level of the seventh cranial nerve were included in data analyses.

2.4. Drugs

Muscimol hydrobromide (Research Biochemical International, Natick, MA) was dissolved in 0.9% sterile saline and fresh solutions of muscimol were prepared on testing days in concentrations of 12.5, 25 or 50 ng/ml. Morphine sulphate (gift of Sabex International, Quebec, Canada) was dissolved in 0.9% sterile saline. Buprenorphine was obtained from the veterinary center of McGill University and was diluted with 0.9% sterile saline. The formalin solution was obtained from diluting 37% formaldehyde with saline to a final concentration of 2% formalin.

2.5. Behavioural testing and injections

Nociception was assessed using the tail immersion version of the tail flick test (Janssen et al., 1963), the hot plate test (Woolfe and MacDonald, 1944) and the formalin test (Dubuisson and Dennis, 1977). For the tail immersion and hot plate tests, rats were briefly handled prior to testing. The

tail immersion test consisted of dipping the tail of the rat into a bath containing water kept at 54 °C. The latency for the rat to remove its tail from the hot bath was recorded. A cut-off of 10 s was imposed to avoid tissue damage. The hot plate apparatus constituted of an aluminum floor (35 × 35 cm), heated to 54 °C, surrounded by clear Plexiglas walls. The latency to display either hind paw licking or flinching/slapping was recorded (Carter, 1991). The animal was removed from the apparatus as soon as a response occurred, or after 30 s. The tail immersion and hot plate tests were carried out in the same session, so that each tail immersion dip was immediately followed by a hot plate test. Baseline latencies were always obtained for each rat prior to drug administration.

The formalin test was carried out in 30 × 30 × 30 cm clear Plexiglas cubicles with a mirror mounted at 45° beneath the floor to allow unobstructed view of the paws. Animals were habituated to the cubicles for 20–30 min/day, for four consecutive days before formalin testing began. Only the second phase of formalin-induced behaviours (persistent inflammatory pain) was rated, and animals were observed at the maximum level of formalin pain, from 25 to 50 min after formalin administration (Abbott et al., 1995). Rating of formalin-induced behaviours was performed according to the method of Dubuisson and Dennis (1977). Different groups of rats received different doses of morphine or buprenorphine, injected s.c. 30 min before behavioural testing. For the experiment involving supraspinal analgesia, morphine was injected i.c.v. over 2 min, in a volume of 10 µl. For this experiment, formalin was injected in the hind paw ipsilateral to the i.c.v. cannula because morphine analgesia elicited i.c.v. has been reported to produce analgesia in the ipsilateral paw, but not in the contralateral paw (Cohen et al., 1984). In all experiments, sterile saline (0.9%) or muscimol was injected 5 min before testing began. The volume of injection was 1 µl over 1 min, and the injection needle remained in place for 1 min to allow for diffusion. A volume of 1 µl ensures that the entire rostral ventromedial medulla is affected (Gilbert and Franklin, 2001), rather than affecting specific nuclei within the rostral ventromedial medulla (Morgan and Fields, 1994).

2.6. Data transformation

Groups were compared using the analysis of variance (ANOVA) procedure, followed by Scheffe post hoc tests. For comparisons between nociceptive tests, data were transformed into maximum percent analgesia (MPA). For the tail immersion test, the E_{min} was 2.9 s (baseline latencies of all animals) and the E_{max} was 10 s, the maximal latency imposed. For the hot plate test, the E_{min} was 6.0 s (baseline latencies of all animals) and the E_{max} was 30 s, the imposed cut-off latency. For the formalin test, the E_{min} was 2.2 (mean pain score of rats treated with 2% formalin and saline in the rostral ventromedial medulla) and the E_{max} was 0, i.e. absence of pain-related behaviours. Estimates of slopes and MPA_{50} of muscimol in each nociceptive test were obtained by jackknifing the data (Abbott et al., 1986; Mosteller and Tukey, 1968; Quenouille, 1956).

3. Results

3.1. Experiment 1. Inactivation of descending fibers from the rostral ventromedial medulla with muscimol: effects on morphine analgesia in the tail immersion, hot plate and formalin tests.

3.1.1. Histology

Fig. 1 shows the microinjection sites of rats included in data analyses of experiment 1 for the tail immersion, hot plate and formalin tests. Out of 36 rats, 33 were included in data analyses and 3 were eliminated because of inaccurate cannula placement.

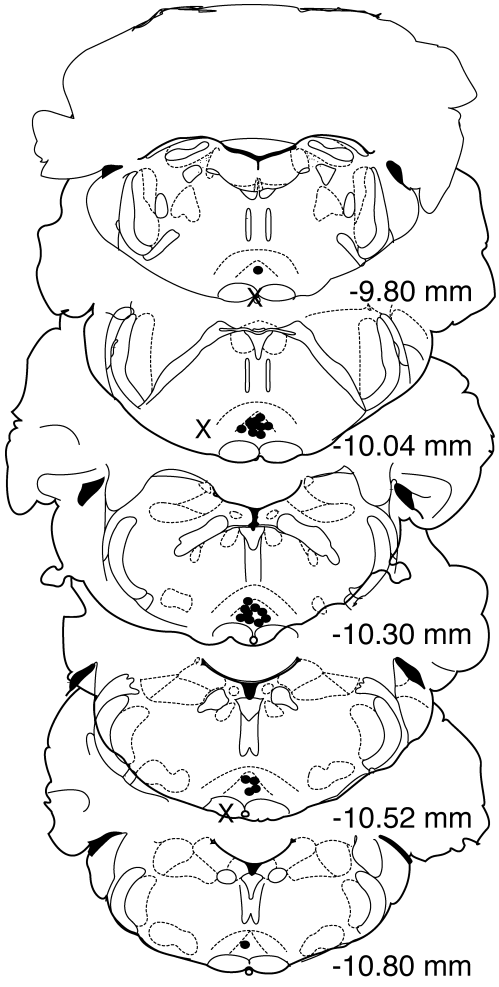

Fig. 1. Location of cannula tips in the rostral ventromedial medulla of rats for experiment 1. Dark dots = accurate placement; X = misplacement. Coordinates are in relation to Bregma. Similar cannula placements were obtained for all four experiments.

3.1.2. Tail immersion test

Fig. 2 shows the effects of muscimol on morphine analgesia in the tail immersion, hot plate and formalin test. In the tail immersion test, baseline tail withdrawal latencies were similar for all groups of rats, which was on average 2.9 s (S.E.M. ± 0.2). Morphine significantly increased tail withdrawal latencies in rats that received saline in the rostral ventromedial medulla, and muscimol dose-dependently decreased morphine analgesia in this test. Compared to latencies of morphine treated rats that received saline in the rostral ventromedial medulla, tail withdrawal latencies of rats injected with muscimol 25 or 50 ng in the rostral ventromedial medulla were significantly shorter, while the dose of 12.5 ng muscimol did not affect morphine analgesia (Scheffe $P=0.05$). Latencies of rats that received systemic morphine plus muscimol 12.5 or 25 ng in the rostral ventromedial medulla were significantly longer than baseline latencies ($F(1,5)=167.5$; $F(1,6)=98.5$, $P<0.05$, respectively), but latencies of rats treated with 50 ng muscimol were not.

3.1.3. Hot plate test

Baseline hot plate response latencies were similar for all rats (average 6 ± 0.2 s, S.E.M.) and morphine significantly increased latencies in rats treated with saline in the rostral ventromedial medulla (see Fig. 2). Muscimol dose-dependently decreased the effect of morphine. Compared to latencies of animals that received saline in the rostral ventromedial medulla, shorter latencies were obtained with all doses of muscimol tested (Scheffe $P=0.05$). Latencies of animals that received muscimol 50 ng in the rostral ventromedial medulla were at baseline, but with 12.5 and 25 ng, latencies were significantly longer than baseline latencies ($F(1,5)=54.0$; $F(1,6)=15.2$, $P<0.05$, respectively).

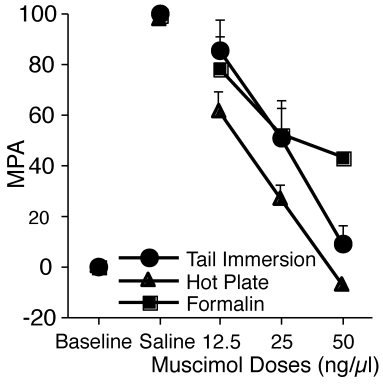

Fig. 2. The effect of muscimol microinjected in the rostral ventromedial medulla on morphine analgesia induced by 5 mg/kg s.c. in the tail immersion, hot plate and formalin tests. Baseline refers to predrug latencies for tail immersion and hot plate tests, and to pain scores generated by 2% formalin for the formalin test. Vertical bars are S.E.M.

Table 1
Parameters estimated by jackknifing the regression equations for the effects of muscimol on morphine analgesia (5 mg/kg) in the three tests

Nociceptive tests	Slope + S.E.M.	MPE_{50} (95% confidence interval)
Formalin	− 29.3 ± 11.9	27.8 (16.9–45.4)
Tail immersion	− 53.6 ± 5.2	24.9 (22.2–28.1)
Hot plate	− 49.4 ± 6.2	15.9 (12.4–20.4)

3.1.4. Formalin test

In rats that received saline in the rostral ventromedial medulla, 5 mg/kg morphine produced 100% analgesia. This analgesia was dose-dependently decreased by muscimol (Fig. 2). Compared to rats that received morphine s.c. and saline in the rostral ventromedial medulla, morphine-treated animals that received muscimol 25 or 50 ng in the rostral ventromedial medulla had significantly higher pain scores (Scheffe $P=0.05$). Pain scores of rats that did not receive morphine (rats treated with formalin and saline in the rostral ventromedial medulla, see baseline) were significantly higher than pain scores of rats treated with morphine and muscimol 12.5, 25 or 50 ng in the rostral ventromedial medulla (Scheffe $P=0.05$), suggesting that none of the doses of muscimol completely abolished morphine analgesia.

3.1.5. Comparison of muscimol dose–response relations in the three pain tests

Table 1 shows the slopes and MPE_{50} for the effects of muscimol on morphine analgesia in the tail immersion, hot plate and formalin tests. The slope of the muscimol dose–response relation was the same in all three tests. The MPE_{50} for muscimol was also similar for each test.

3.2. Experiment 2. Morphine dose–response relations when descending fibers from the rostral ventromedial medulla are inactivated

This experiment examined the dose–response relation for morphine when descending fibers are inactivated. Typically, the dose–response relation for morphine is asymptotic so that morphine produces dose-dependent analgesia until it reaches 100%. When descending fibers are inactivated, it is not known whether the analgesic effect of morphine is decreased by a constant proportion (parallel shift) or whether high doses of morphine can overcome the block induced by inactivation of descending fibers (change in slope).

3.2.1. Tail immersion test

Fig. 3A illustrates the morphine dose–response relation after microinjection of saline or muscimol (50 ng) in the rostral ventromedial medulla. After microinjection of saline in the rostral ventromedial medulla, the analgesic effect of morphine was dose-dependent. More analgesia was

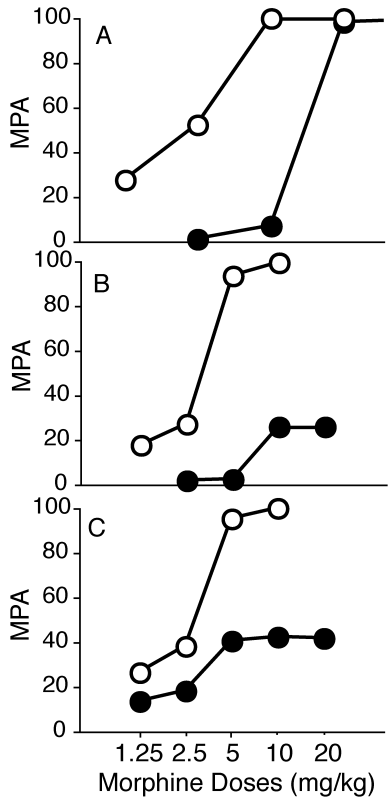

Fig. 3. Dose–response relations for systemic morphine after microinjection of saline (clear circle) or 50 ng muscimol (filled circle) in the rostral ventromedial medulla in the (A) tail immersion, (B) hot plate and (C) formalin test. Morphine was injected s.c. 30 min before testing and saline or muscimol was injected 5 min before testing.

observed with increasing morphine doses from 1.25 to 5 mg, but 10 mg did not produce more analgesia than 5 mg/kg (Scheffe $P=0.05$). After muscimol in the rostral ventromedial medulla, the analgesic effect of morphine was also dose-dependent. Comparison of the morphine dose–response relations revealed that muscimol significantly increased the slope of the morphine dose–response relation (slope and S.E.M.: saline: 51.7 ± 4.2; muscimol: 70.1 ± 4.5, $t=2.9$, $P<0.05$). The maximum analgesic efficacy of morphine was the same for saline- and muscimol-treated rats: morphine produced 100% analgesia.

3.2.2. Hot plate test

In the hot plate test, muscimol decreased morphine analgesia, and high doses of morphine failed to reinstate full analgesia (Fig. 3B). The analgesic effect of morphine was dose-dependent in rats that received saline in the rostral ventromedial medulla but compared to the dose of 5 mg/kg, 10 mg/kg did not produce a further increase in analgesia (Scheffe $P=0.05$). After microinjection of muscimol in the rostral ventromedial medulla, the analgesic effect of morphine was also dose-dependent but muscimol significantly reduced the slope of the morphine dose–response relation (slope and S.E.M.: saline: 64.2, ± 5.6; muscimol: 22.4 ± 2.9, $t=6.6$, $P<0.05$). While the analgesic efficacy of morphine was almost 100% at 5 mg/kg with saline in the rostral ventromedial medulla, the effect of morphine reached asymptote with the dose of 10 mg/kg in muscimol-treated rats. After muscimol in the rostral ventromedial medulla, the analgesic effect of 20 mg/kg was not greater than that of 10 mg/kg (Scheffe $P=0.05$) and was only 25% of the maximum possible analgesia.

3.2.3. Formalin test

After injection of saline in the rostral ventromedial medulla, the analgesic effect of morphine was dose-dependent but the dose of 10 mg did not produce more analgesia than 5 mg/kg (Scheffe $P=0.05$). After muscimol in the rostral ventromedial medulla, the analgesic effect of morphine was also dose-dependent though the slope of the morphine dose–response relation was reduced (slope and S.E.M.: saline: 47.8 ± 6.3; muscimol: 18.7 ± 6.0, $t=3.3$, $P<0.05$). After saline in the rostral ventromedial medulla, analgesia reached 100% with a dose of 5 mg. The maximum effect of morphine after muscimol in the rostral ventromedial medulla reached asymptote approximately at 40%. The effects of 5, 10 and 20 mg/kg morphine did not differ from one another (Scheffe $P=0.05$).

3.2.4. Comparison of the morphine dose–response relations in the three pain tests

After microinjection of saline in the rostral ventromedial medulla, the slopes, the MPA_{50} and the MPA_{100} for the morphine dose–response relations were the same in all three tests (see Table 2A). However, after microinjection of muscimol in the rostral ventromedial medulla (see Table 2B), the slope of the morphine dose–response relation was significantly steeper in the tail immersion than in the two other tests. The efficacy of morphine was much greater in the tail immersion than in the two other tests. Also, the maximum analgesic efficacy of morphine was significantly higher in the formalin test than in the hot plate test.

3.3. Experiment 3. Supraspinal morphine analgesia when descending fibers from the rostral ventromedial medulla are inactivated

Since inactivation of descending fibers did not abolish morphine analgesia, the previous experiments indicated that

Table 2A
Comparison of slopes, MPA_{50} and MPA_{100} for morphine after microinjection of saline in the rostral ventromedial medulla in the three nociceptive tests

Nociceptive tests	Slope + S.E.M.	MPA_{50} (95% CI)	MPA_{100}
Formalin test	47.8 ± 6.3	2.3 (1.9–2.8)	96.4%
Tail immersion test	51.7 ± 4.3	2.1 (1.8–2.3)	99.9%
Hot plate test	64.2 ± 5.6	2.8 (2.5–3.2)	96.3%

Table 2B
Comparisons of slopes, MPA_{50} and MPA_{100} for morphine after microinjection of muscimol (50 ng) in the rostral ventromedial in the three nociceptive tests

Nociceptive tests	Slope + S.E.M.	MPA_{50} (95% CI)	MPA_{100}
Formalin test	18.7 ± 6.0	N.A.	43.0%[a]
Tail immersion test	70.1 ± 4.6[b]	5.5 (4.9–6.1)	99.8%[b]
Hot plate test	22.4 ± 2.9	N.A.	25.2%

N.A.: Not available, maximal effect below MPA_{50}.
CI = Confidence interval.
[a] MPA_{100} for formalin significantly higher than for hot plate test.
[b] Slope or MPA_{100} for tail immersion test significantly higher than in the hot plate and formalin tests.

morphine analgesia is not entirely dependent on descending fibers from the rostral ventromedial medulla. Therefore, in experiment 3, we investigated the analgesic effect of morphine elicited i.c.v. when descending fibers from the rostral ventromedial medulla are inactivated by muscimol. As can be seen in Fig. 4, injection of morphine i.c.v. produced a dose-dependent analgesia in rats microinjected with saline in the rostral ventromedial medulla. Microinjection of muscimol in the rostral ventromedial medulla decreased morphine analgesia elicited i.c.v. and reduced the slope of the morphine dose–response relation ($t = 7.5$, $P < 0.05$), such that the slope of the morphine line was not significantly greater than 0. The pain scores of rats treated with morphine i.c.v. plus muscimol in the rostral ventromedial medulla were within the range of pain scores of rats treated with 2% formalin and saline in the rostral ventromedial medulla.

3.4. Experiment 4. Buprenorphine analgesia when descending fibers from the rostral ventromedial medulla are inactivated

To extend the results obtained with morphine to another opioid, we evaluated the contribution of descending fibers from the rostral ventromedial medulla to buprenorphine analgesia. Buprenorphine was chosen because it is an opioid with strong analgesic activity and a pharmacological profile different from that of morphine. Buprenorphine is classified as a mixed agonist–antagonist acting as a partial mu agonist (Dum and Herz, 1981) and a kappa antagonist (Leander, 1987). As can be seen in Fig. 5, buprenorphine produced dose-dependent analgesia after saline in the rostral ventromedial medulla in all three tests. Note that in the formalin test, higher doses of buprenorphine were required to obtain the same magnitude of effect than in the two other tests. In the hot plate test, the buprenorphine dose–response curve appeared biphasic, although the dose of 200 mg did not produce less analgesia than 100 mg/kg. The biphasic dose–response curve for buprenorphine in the hot plate test was confirmed by testing four additional rats with the dose of 400 mg/kg which produced 25% analgesia (data not shown). It is interesting to note that after the saline treatment, the maximal analgesic efficacy of buprenorphine was significantly lower in the hot plate than in the tail immersion and formalin tests.

After microinjection of muscimol, buprenorphine produced some analgesia in the tail immersion test (Scheffe $P = 0.05$) but the maximum analgesic efficacy of buprenorphine was greatly reduced (MPA_{100} and S.E.M.: saline: 95.9 ± 2.3; muscimol: 20.5 ± 6.1, $t = 12.2$, $P < 0.05$). Thus, muscimol significantly reduced the slope of the buprenorphine dose–response relation in the tail immersion test.

In the hot plate test, buprenorphine did not produce analgesia after microinjection of muscimol in the rostral ventromedial medulla. Muscimol reduced the slope of the buprenorphine dose–response relation ($t = 7.6$, $P < 0.01$) to 0. A similar pattern of findings was observed in the formalin test after microinjection of muscimol in the rostral ventromedial medulla: buprenorphine failed to produce analgesia. The reduction in slope of the buprenorphine dose–response relation was significant ($t = 11.7$, $P < 0.05$).

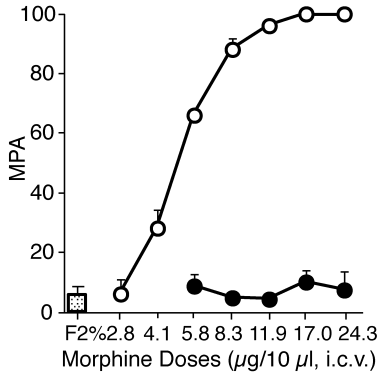

Fig. 4. Dose–effect relation for i.c.v. morphine after microinjection of saline (clear circle) or 50 ng muscimol (dark circle) in the rostral ventromedial medulla in the formalin test. F2% refers to pain scores of control rats treated with 2% formalin and saline in the rostral ventromedial medulla ($n = 6$). Vertical bars are S.E.M.

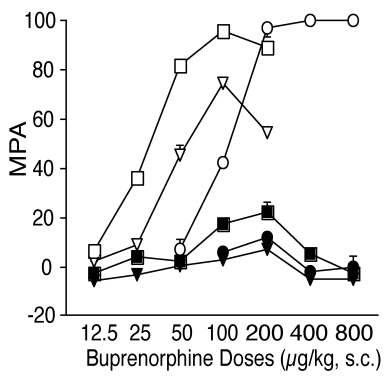

Fig. 5. Dose–effect relation for systemic buprenorphine after microinjection of saline or 50 ng muscimol in the rostral ventromedial medulla in the tail immersion, hot plate and formalin tests. Saline—clear symbols; muscimol—dark symbols. Square—tail immersion test; triangle—hot plate test; circle—formalin test. Vertical bars are S.E.M.

4. Discussion

The first experiment examined the effects of muscimol (12.5–50 ng) microinjected into the rostral ventromedial medulla on the analgesic effect of 5 mg/kg systemic morphine in the tail immersion, hot plate and formalin tests. This dose of morphine produced 100% analgesia in all three tests. Muscimol in the rostral ventromedial medulla dose-dependently decreased morphine analgesia in all three tests. The highest dose of muscimol (50 ng) abolished morphine analgesia in the tail immersion and hot plate tests, but only removed 60% of the analgesia in the formalin test.

This latter observation contrasts with a number of studies in which morphine analgesia in the formalin was not affected by dorsolateral funiculus or lesions of the rostral ventromedial medulla (Abbott and Melzack, 1982; Abbott et al., 1982; Ryan et al., 1985). However, the present findings agree with the results of a recent study in which the analgesic effect of morphine was reduced following lesions of the dorsolateral funiculus (Abbott et al., 1996). In that study, it was argued that the decreased analgesic efficacy of morphine could be in part due to an increased sensitivity to pain induced by dorsolateral funiculus lesions (Abbott et al., 1996). However, it is unlikely that an increased sensitivity to formalin pain accounted for the reduced effect of morphine in the present study since we have shown that rostral ventromedial medulla microinjection of muscimol (50 ng) does not significantly affect pain scores induced by 2% formalin (Gilbert and Franklin, 2001).

In the present study, the analgesic effect of morphine in the formalin test was not as sensitive to the action of muscimol as it was in the tail immersion and hot plate tests. This observation may indicate that the analgesic action of morphine in the formalin test involves other sites of action, independent of fibers originating in the rostral ventromedial medulla. Experiment 3 tested the hypothesis that the 40% residual of systemic morphine analgesia after inactivation of descending fibers in the formalin test was due to a supraspinal action. However, after microinjection of morphine i.c.v. and inactivation of descending fibers from the rostral ventromedial medulla with muscimol (50 ng), no significant analgesia was obtained with doses up to five times the MPA_{50} of controls (Fig. 4). Likewise, the analgesic effect of the mu agonist Tyr-D-Ala-Gly-*N*-Methyl-Phe-Gly-ol (DAMGO) administered i.c.v. is completely abolished by lesion of the dorsolateral funiculus (Gogas et al., 1991). Lesions of the dorsolateral funiculus also eliminate the suppressive effect of i.c.v. DAMGO on formalin-induced c-*fos* expression in the dorsal horn of the spinal cord (Gogas et al., 1991). Together, these findings strongly support the view that the supraspinal action of morphine is to trigger descending inhibition from the rostral ventromedial medulla. Also supporting this idea are reports showing that supraspinal administration of morphine inhibits the firing of spinal cord dorsal horn neurons responding to noxious stimuli (Bennet and Mayer, 1979; Du et al., 1984). The fact that intracerebral microinjection of morphine attenuates the spinally mediated tail flick reflex is also consistent with this notion (Jensen and Yaksh, 1986).

Experiment 2 examined the analgesic effect of morphine (1.25–20 mg/kg) to see whether high doses of morphine could overcome the block induced by microinjection of muscimol in the rostral ventromedial medulla. In the hot plate and formalin tests, the dose–response relations of morphine after muscimol in the rostral ventromedial medulla reached a ceiling at 25% and 40% analgesia, respectively. Only in the tail immersion test did a higher dose of morphine completely reverse the blocking effect of muscimol.

These results raise the questions of what mechanisms mediate the partial analgesic effect of high doses of morphine and why do the dose–response relations for morphine analgesia in the three tests differ after rostral ventromedial medulla muscimol when they are very similar without muscimol. The possibility that the tests simply differ in sensitivity to morphine or muscimol, the tail immersion being the least sensitive, can be rejected. The tests showed similar sensitivity to morphine given alone, and in all three tests, the steeply rising portion of the dose–response relation lays between 2.5 and 5 mg/kg. Furthermore, experiment 1 showed the potency of muscimol in the three tests was the same. If anything, it was the formalin test that showed the most resistance to muscimol, not the tail immersion test.

A more likely explanation is that the reinstatement of analgesia induced by high doses of 10 and 20 mg/kg reflects a spinal or peripheral effect of morphine. After systemic administration, morphine is evenly distributed throughout the brain and spinal cord (Matos et al., 1995), and inactivation of descending fibers should not prevent the pre- and post-synaptic action of morphine in the spinal cord. The finding that inactivation of descending fibers completely abolished morphine analgesia (5 mg/kg) in the hot plate and tail immersion tests suggests that the spinal effect of morphine is minimal with systemic injection of a moderate dose in rats. Likewise, morphine analgesia in the 3–7-mg/kg range is eliminated by bilateral injection of quaternary naloxone into the posterior hypothalamic area (Manning and Franklin, 1998), lesions of the amygdala (Manning and Mayer, 1995b) or lesions of the dorsolateral funiculus (Basbaum et al., 1977). Our findings are also consistent with earlier studies which suggest that transection of the spinal cord reduces the potency and efficacy of morphine (Bonnycastle et al., 1953; Irwin et al., 1950). After rostral ventromedial medulla muscimol, the efficacy of morphine appeared greater in the tail withdrawal test than in the other two tests. This may be because the tail flick reflex is spinally mediated. However, the fact that spinal transection reduces the efficacy of systemic morphine in the tail flick test (Irwin et al., 1950) is against this interpretation. An alternative explanation is that a high dose of morphine (10 mg/kg) may have interfered with responding in the tail immersion test. It is known that morphine can induce rigidity of the tail in rats

(Katz, 1979), a phenomenon called "straub tail". During testing, it was noticed that the tail of the rats was elevated and rigid. It was also noted that the rats would struggle instead of flicking their tail, suggesting that the animals were reacting to the noxious stimulation but could not perform the required response. The unusual shape of the dose–response curve also supports this explanation. The reversal of muscimol occurred suddenly with 10 mg/kg, rather than being a dose-dependent effect (see Fig. 3). Thus, the interpretation of the reversal of the effect of muscimol by 10 mg/kg morphine in the tail immersion test is problematic.

In the formalin test, microinjection of muscimol in the rostral ventromedial medulla did not appear to change the potency of morphine, even though the efficacy was greatly reduced. In muscimol or saline-treated rats, the analgesic effect reached asymptote at 5 mg/kg. Likewise, in experiment 1, the highest behaviourally selective dose of muscimol (50 ng) reduced morphine analgesia to 40% in the formalin test, but eliminated analgesia in the other two tests. These findings do not support the idea that a spinal action of morphine fully accounts for the 40% remaining analgesia in this test. The residual analgesic effect in the formalin test was maximal at a dose (5 mg/kg) that was completely blocked by muscimol in the tail immersion and hot plate tests. It might be possible that formalin induces neurochemical changes that promote the spinal effect of morphine, but indirect evidence does not support this hypothesis. The second phase of formalin pain involves wind up and/or central sensitization, which are characterized by increased excitability of neurons and prolonged discharge following activation of primary afferent fibers (Sorkin and Wallace, 1999). Several lines of evidence indicate that wind up and central sensitization are initiated by the co-release of substance P and excitatory amino acids (Sorkin and Wallace, 1999). However, inhibition of substance P release does not contribute significantly to the analgesic effect of morphine after either systemic or intrathecal administration (Trafton et al., 1999). Likewise, administration of mu-opioid receptor agonists in the spinal cord does not eliminate the release of glutamate induced by formalin (Buerkle et al., 1998). Moreover, morphine has lower potency and efficacy against long-term potentiation mediated by *N*-methyl-D-aspartate (NMDA) of dorsal horn neurons than against nonpotentiated baseline responses of dorsal horn neurons (Rygh et al., 2000). Thus, the neurochemical changes induced by formalin do not offer a basis for an enhanced spinal effect of morphine.

The evidence against the predominance of a spinal action, together with the failure to demonstrate a supraspinal action of morphine after microinjection of muscimol in the rostral ventromedial medulla (experiment 3), suggest that the residual analgesic effect (40%) of morphine in the formalin test occurs neither in the brain nor in the spinal cord. The possibility that morphine has peripheral effects in the formalin test is supported by previous studies showing a peripheral action of morphine in the formalin test after systemic injection (Taylor et al., 1997; Taylor et al., 2000) or after local injection into the affected paw (Hong and Abbott, 1995). It has been demonstrated that the presence of inflammation is crucial for the manifestation of peripheral effects of opioids since opioid receptors on axons (Coggeshall et al., 1997) and peripheral terminals (Stein et al., 1990) of peripheral sensory axons are not functional in normal, uninjured tissue (Stein et al., 1988). Since the second phase of formalin is characterized by an acute inflammatory reaction (Omote et al., 1998), the inflammatory response induced by formalin may promote the peripheral antinociceptive effect of morphine, which will be unimpaired by inactivation of descending fibers from the rostral ventromedial medulla.

The effects of microinjection of muscimol in the rostral ventromedial medulla on buprenorphine analgesia provided some additional support for these interpretations, though the data obtained with high doses of buprenorphine must be interpreted with caution because high doses of buprenorphine were less active than lower doses. As previously reported, 200 mg buprenorphine completely suppressed pain responses in the formalin test (Abbott and Bonder, 1997). Buprenorphine analgesia was completely abolished by microinjection of muscimol in the rostral ventromedial medulla in this test, suggesting that the analgesic effect of buprenorphine depends on brainstem descending fibers. Consistent with the view that spinal and peripheral mechanisms explain the residual analgesia produced by morphine in the formalin test, buprenorphine has only weak spinal and peripheral actions. Previous studies have found that intrathecal buprenorphine fails to block the transmission of nociceptive inputs in the spinal cord (Dickenson et al., 1990). Moreover, the analgesic effect of buprenorphine after i.t. injection is very weak in rats: antinociception is obtained with a dose of 10 mg i.t., which exceeds the dose required parentally (Bryant et al., 1983). Thus, evidence accumulated to date does not favor a spinal action for the analgesic effect of buprenorphine and the present data confirm that the analgesic effect of buprenorphine is mediated via rostral ventromedial medulla descending fibers.

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