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Systemic and spinal administration of the *mu* opioid, remifentanil, produces antinociception in amphibians

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Abstract

Remifentanil is a relatively new opioid analgesic related to the fentanyl family of *mu* opioid receptor agonists and is used clinically for its unique property of having an ultra-short duration of action. However, there is little preclinical data on the analgesic (antinociceptive) effects of remifentanil and none obtained in non-mammalian animal models. The antinociceptive effects of remifentanil were assessed by using the acetic acid test in amphibians. Systemic and spinal administration of remifentanil was made by subcutaneous and intraspinal injections in the Northern grass frog, *Rana pipiens*. After administration, remifentanil produced dose-dependent and long-lasting antinociceptive effects which persisted for five hours after systemic administration but gave a shorter duration of action after spinal delivery. The antinociceptive effects of remifentanil were significantly blocked by pretreatment with systemic naltrexone. Systemic and spinal administration of remifentanil produced log dose–response curves which yielded ED_{50} values of 7.1 nmol/g and 3.2 nmol/animal respectively. The relative antinociceptive potency of remifentanil compared to other opioids administered to amphibians is similar to that found in mammalian models.

Keywords

Remifentanil; Analgesia; Opioid; *Mu* opioid receptor; *Rana pipiens*

1. Introduction

Remifentanil is a unique *mu* opioid analgesic with an ultra-short duration of action due to its inactivation by non-specific esterases in plasma and tissues (Michelsen and Hug, 1996). Marketed in Europe and in the United States as Ultiva®, remifentanil is used broadly in clinical anesthesiology; employed in obstetrics, pediatrics, cardiology and for a variety of surgical and outpatient procedures (Beers and Camporesi, 2004; Cohen and Royston, 2001; Van de Velde, 2005). Remifentanil is a potent opioid analgesic, as much as 60-times more potent than alfentanil but with an elimination half-life of about 10 min in humans (Dershwitz et al., 1995; Hoke et al., 1997).

Surprisingly, there is little data on the analgesic or antinociceptive effects of remifentanil in nonhuman animals. Early studies established the *mu* opioid receptor selectivity of remifentanil in tissue assays and the relative analgesic potency of systemically administered remifentanil in rodents (James et al., 1991). These studies showed that remifentanil was about equipotent to fentanyl and more potent than sufentanil or alfentanil. The analgesic potency of remifentanil and its short duration of action were confirmed after central

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administration by the spinal route using intrathecal catheters in rats (Buerkle and Yaksh, 1996). Remifentanil also inhibited nociceptive behavior following the hindpaw injection of formalin in rodents (Buerkle et al., 1998; Taylor et al., 1997) and the antinociceptive effects of remifentanil were studied in a rabbit model of anesthesia and analgesia (Hayashida et al., 2003a,b).

As part of an overall goal of testing novel *mu* opioid analgesics for use in a spinal opioid tolerance model in amphibians, we evaluated the nociceptive effects of systemic and spinal remifentanil in the Northern grass frog, *Rana pipiens*. This amphibian model is a well-developed alternative or adjunct model for the testing of opioid analgesics using non-mammalian vertebrates (Stevens, 1992, 2004). The algesiometric assay used in amphibians is called the acetic acid test and consists of monitoring the response of the animal to drops of log-spaced concentrations of acetic acid placed on the thigh of the unanesthetized animal. The nociceptive threshold obtained using the acetic acid test is robust and stable over a period of hours and days, and the wiping response used in the behavioral assay has not been observed in the absence of noxious stimuli. Thus, the acetic acid test appears to be specific for assessing a brief noxious event (Pezalla, 1983; Stevens et al., 1994). Previous work showed that systemic, supraspinal, and spinal administration of a series of opioid analgesics to amphibians produced antinociceptive effects on the acetic acid test with a rank order of relative potency correlated to that found in mammalian species and in humans (Stevens et al., 1994; Stevens, 1996; Stevens and Rothe, 1997). Given the lack of information on remifentanil's action in non-mammalian vertebrates and the ongoing efforts to develop the amphibian model, the present study sought to obtain data on the time course, naltrexone antagonism, and dose–response characteristics of the antinociceptive effects of remifentanil after systemic and spinal administration in *R. pipiens*.

2. Materials and methods

All procedures were approved by the Institutional Animal Use Committee (IACUC) and adhere to the National Institutes of Health (U.S.A.) and the European Community guidelines on the use of animals.

2.1. Animals, drugs, and experimental procedures

Northern grass frogs, *R. pipiens* (mean weight, 28.0 g), were obtained from a commercial supplier (Sullivan Co., Inc, Nashville, TN, USA). Frogs were held in flow-through, stainless-steel aquaria in groups of 48 immediately after arrival and were fed live crickets 3 times a week.

Drugs used were remifentanil hydrochloride (3-[4-meth-oxycarbonyl-4-[(1-oxopropyl) phenylamino]-1-piperidine] propanoic acid methyl ester, Abbott Laboratories, North Chicago, IL, USA) and naltrexone hydrochloride (Sigma Chemical Co., St. Louis, MO. USA). Drugs were dissolved in sterile 0.9% saline to yield nanomole/gram body weight doses of the free base. Remifentanil was administered at doses of 1, 3, 10, and 30 nmol/g by the systemic route and given by intraspinal injection at doses of 3, 10, 30, 100 and 300 nmol/frog. Systemic administration was made by bolus injection into the dorsal lymph sac at a volume of 10 μl/g body weight (Stevens et al., 1994). Spinal administration was done using a Hamilton microsyringe and was made between the articulation of the lumbar vertebrate in a volume of 5 μl (Stevens, 1996). Saline-injected control animals were co-run with remifentanil and naltrexone antagonist experiments, and there were no significant changes from baseline values (data not shown). For opioid antagonism experiments, systemic naltrexone (100 nmol/g) or saline was administered 1 h before the systemic or spinal administration of remifentanil.

2.2. The acetic aid test and data analysis

At least two days before their use in any experiment, the frogs were transferred into the laboratory and placed in individual plastic cages with mesh tops and sufficient water. On experiment days, water was lowered to expose the dorsum of the thigh for acetic acid testing and 1 h later the baseline nociceptive threshold was obtained. The acetic acid test was used to assess the nociceptive threshold in frogs as fully described elsewhere (Pezalla, 1983). Briefly, 10 solutions of acetic acid were serially diluted (1:2, water:acid) from glacial acetic acid (17.5 M) to give log-spaced dilutions and labeled with the lowest number being equal to the lowest concentration of acetic acid. The nociceptive threshold was determined by placing a drop of acetic acid on the dorsal surface of the frog's thigh. Testing began with the lowest concentration of acid and proceeded stepwise on alternative hind limbs until the nociceptive threshold was reached. The nociceptive threshold was defined as the code number of the lowest concentration of acid, which caused the frog to behave and wipe the treated leg with either hind limb. To prevent tissue damage, the drop of acetic acid was washed off immediately with a gentle stream of water once the animal responded or after 5 s if the animal failed to respond. In some instances, an nociceptive threshold of 11 (cutoff value) was recorded; this was only the case if the frog failed to respond to the highest concentration of acetic acid (10, glacial acetic acid).

To generate a time–response curve, baseline nociceptive threshold was obtained 1 h before and at post-treatment times of 15, 30, 60, 180 and 300 min after remifentanil administration. Peak maximum percent effect (MPE) values of individual animals from the time–response data were pooled for each dose to construct the dose–response curves. MPE was obtained from the raw nociceptive threshold (NT) values by the formula below:

$$\text{MPE}=\frac{\text{Post}-\text{treatment NT}-\text{Baseline NT}}{\text{Cutoff value (11)}-\text{Baseline NT}}\times 100$$

MPE data were entered into the PHARM/PCS (v.4) program to calculate the dose producing a 50% effect (ED_{50}) and the 95% confidence intervals. A Student's *t*-test was used to assess the effects of naltrexone pretreatment vs. saline pretreatment on remifentanil administration. Treatment groups consisted of 6–12 animals and animals were used only once.

3. Results

At the doses reported, remifentanil did not produce any signs of adverse effects following systemic or spinal administration. Animals exhibited normal righting and corneal reflexes and remifentanil at times of testing and animals had NT values that returned to baseline levels 24 h after administration of remifentanil (data not shown).

3.1. Time course of the antinociceptive effects of systemic and spinal remifentanil

Fig. 1 shows the time course of the antinociceptive effects of remifentanil after systemic (A) and spinal administration (B). A dose-dependent increase in antinociceptive effects (MPE) of systemic remifentanil was observed at 30 min after 1, 3, 10, and 30 nmol/g remifentanil. The antinociceptive effects of these four doses of remifentanil remained constant over the 300 min (5 h) time course of the experiment. Following spinal administration of remifentanil, dose-dependent antinociceptive effects were noted at the first post-treatment test point (15 min). However, remifentanil at the highest doses of 100 or 300 nmol/frog showed a decrement of antinociceptive effects by 300 min after spinal administration.

3.2. Effect of naltrexone pretreatment on the antinociceptive effects of remifentanil

The opioid receptor antagonist naltrexone (100 nmol/g, s.c.) administered 1 h before the systemic administration of remifentanil (30 nmol/g) resulted in a significant reduction of the antinociceptive effect as shown by comparison of the saline-pretreated group and the naltrexone pretreated animals (Fig. 2A). The antinociception produced by spinal administration of remifentanil (100 nmol/frog) was also blocked by naltrexone pretreatment, as shown in Fig 2B.

3.3. Relative potency of remifentanil in amphibians

Log dose–response curves of the antinociceptive effect of remifentanil following systemic and spinal administration in amphibians are shown in Fig. 3. Systemic remifentanil gave an ED_{50} of 7.07 nmol/g (Fig. 3A), similar to that of levorphanol and fentanyl and more potent than morphine (from previous studies, Stevens et al., 1994). Spinal administration of remifentanil yielded an ED_{50} value of 3.21 nmol/frog (Fig. 3B), which was less potent than the *mu* opioid receptor agonists fentanyl, DAMGO ([D-Ala^2,NMePhe^4,Gly-ol]-enkephalin), and morphine (from previous studies, Stevens, 1996). As shown in Table 1, remifentanil was significantly more potent (12.2 times) than morphine, slightly less potent than fentanyl and equipotent to levorphanol after systemic administration. Following spinal administration, remifentanil was nearly equipotent to morphine and fentanyl, but significantly less potent than DAMGO.

4. Discussion

The present results demonstrate that remifentanil produces a potent and dose-dependent antinociceptive effect after systemic and spinal administration in an alternative pain research model using amphibians.

4.1. Time course of remifentanil antinociception in amphibians

Following systemic administration into the dorsal lymph sac, remifentanil produced a dose-dependent antinociceptive effect that was apparent at the time of the first post-treatment algesiometric test (Fig. 1A). The antinociceptive effect was persistent for at least 5 h following administration of the three highest doses of remifentanil, with animals returning to normal baseline thresholds the next day. In mammalian studies, systemic administration of remifentanil by intraperitoneal injection in rodents gave a short duration of action, in the range of 10–15 min (Buerkle and Yaksh, 1996). However, systemic administration of all opioid receptor agonists in amphibians produces a surprisingly long duration of action, which may be due to the depot-like injection into the dorsal lymph sac with slow release of analgesic into the systemic circulation, low body temperature and sluggish circulation, and decreased metabolic activity as discussed earlier (Stevens et al., 1994). There is no evidence of rapid metabolism of remifentanil following systemic administration in amphibians, although the pharmacokinetic differences just mentioned above may mask such an effect.

Following spinal administration of remifentanil in amphibians, peak antinociceptive effects are noted at 15 min after intraspinal administration for the four highest doses of remifentanil, with the lowest dose showing a slightly longer time to peak effect (Fig. 1B). However, the last time point of testing (5 h after administration) shows that three highest doses of remifentanil have decreased to 30–40% MPE. This is in contrast to previous studies of a number of *mu*, *delta*, and *kappa*-selective opioid receptor agonists administered via the spinal route in amphibians whereby all agents and doses maintained significant antinociceptive effects and flat effect curves throughout the time course of testing (Stevens, 1996). This may be evidence of a more rapid metabolism of remifentanil than other opioid analgesics after spinal administration in amphibians. The amphibian CNS and peripheral

tissues are replete with esterase enzymes (Contestabile, 1976;Gabriel and Budai, 1992;Hardwick and Hebb, 1956) as is the case for all vertebrate animals tested (Chuiko et al., 2003;Fossi et al., 1992;Sanchez et al., 1997).

4.2. Naltrexone antagonism of remifentanil antinociception in amphibians

The putative action of remifentanil as an opioid receptor agonist was assessed in amphibians by systemic pretreatment of animals with the general opioid receptor antagonist, naltrexone. In previous studies, naltrexone at this dose of 100 nmol/g, s.c. given 1 h before systemic administration of ten different *mu* and *kappa* selective opioid analgesic agents (Stevens et al., 1994) or given before the spinal administration of twelve different *mu*, *delta*, or *kappa* selective opioid receptor agonists significantly blocked the antinociceptive effects of subsequent agonist administration (Stevens, 1996). As shown in Fig. 2A, pretreatment with naltrexone significantly blocked the antinociceptive effect of systemic remifentanil (30 nmol/g. s.c.). Remifentanil administration by the spinal route (100 nmol/frog, i.s.) was also blocked by naltrexone pretreatment (Fig. 2B). While these data demonstrate opioid receptor activation in amphibians mediates remifentanil antinociception, the use in amphibian studies of opioid receptor antagonists that are highly selective for each type of opioid receptor in mammals did not show convincing type-selectivity in behavioral and binding studies in amphibians (Newman et al., 2000a,b; Stevens and Newman, 1999). However, consistent findings from in vivo and in vitro studies in mammals (see Introduction) demonstrate that remifentanil is highly selective for the *mu* opioid receptor it is likely that remifentanil produces its effect predominantly by interaction of the species ortholog of the *mu* opioid receptor protein in amphibians (see below).

4.3. Relative antinociceptive potency of remifentanil in amphibians

The dose–response curves of the antinociceptive effects of remifentanil following systemic and spinal administration in amphibians are shown in Fig 3. For comparison, plotted also are the antinociceptive effects of morphine and related opioid receptor agonists in amphibians from previous larger studies (Stevens et al., 1994;Stevens, 1996). Systemic remifentanil gave a dose–response curve that did not differ in potency from fentanyl or levorphanol (overlapping 95% confidence intervals, see Table 1) but was significantly more potent than morphine. The ED_{50} of systemic remifentanil was 7.1 nmol/g compared to 86.3 nmol/g for morphine, making remifentanil about 12 times more potent than morphine. In rodent studies, remifentanil was also more potent than morphine and about equipotent to alfentanil following systemic administration (Buerkle and Yaksh, 1996). Following spinal administration, remifentanil had an ED_{50} value of 3.2 nmol/animal which was not significantly different than morphine (2.3 nmol/animal) or fentanyl (0.9 nmol/animal) but remifentanil was significantly less potent than the enkephalin analog, DAMGO (Table 1). Intrathecal administration of remifentanil in rodents found that remifentanil was significantly more potent than morphine (Buerkle and Yaksh, 1996). The overall relative potency between a number of opioid receptor agonists is well-correlated in rodents and amphibians following systemic, spinal, and intracerebroventricular administration (Stevens et al., 1994;Stevens, 1996;Stevens and Rothe, 1997). However, recent preliminary data following the cloning and characterization of amphibian *mu*, *delta*, and *kappa* opioid receptor proteins show that opioid receptor sequences are more similar among themselves in amphibians compared to sequences from mammals (Stevens, 2003,2004). These findings suggest that opioid receptor type-selectivity may be developed less in earlier-evolved vertebrates and that, in general, greater type-selectivity among close members of the same receptor family is a driving force of molecular evolution.

In summary, remifentanil administration produced potent and dose-dependent antinociceptive effects following systemic and spinal administration in amphibians. The

antinociceptive effects of remifentanil were significantly blocked by pretreatment of naltrexone and the relative potency of remifentanil compared to other opioids is similar to that found in mammalian models. The decrement of antinociceptive effects of remifentanil following spinal administration may reflect more rapid metabolism by this route compared to other opioid agents given in previous amphibian studies. Remifentanil may be a suitable choice as a *mu* opioid analgesic to continue studies of spinal opioid tolerance in the amphibian model.

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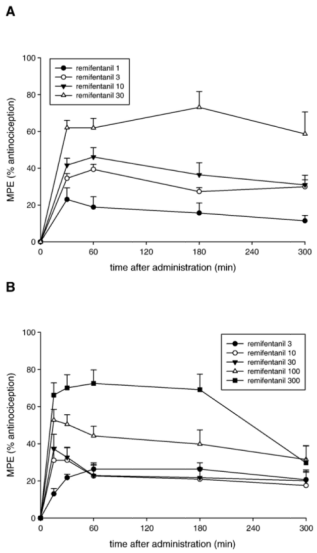

Fig. 1.

Time course of the antinociceptive effect of remifentanil following systemic (A) or spinal (B) administration in amphibians. Doses used are given in boxes on the plots in nmol/g for systemic and nmol/frog for spinal administration. Data points are plotted as MPE+S.E.M. for *N*=6–12 animals per dose.

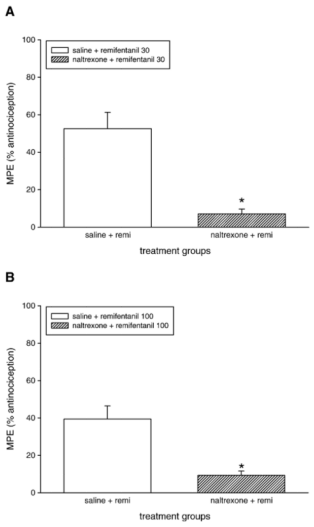

Fig. 2.

Effect of naltrexone pretreatment on the antinociceptive effect of remifentanil following systemic (A) or spinal (B) administration in amphibians. Systemic administration of saline (filled bars) or naltrexone (100 nmol/g s.c., hatched bars) was given 60 min before systemic or spinal administration of remifentanil (doses given on plot). Data are plotted as the mean MPE+S.E.M. for six animals per treatment group. Asterisks (*) denote significant difference from saline-pretreated groups ($P<0.05$, Student's *t*-test).

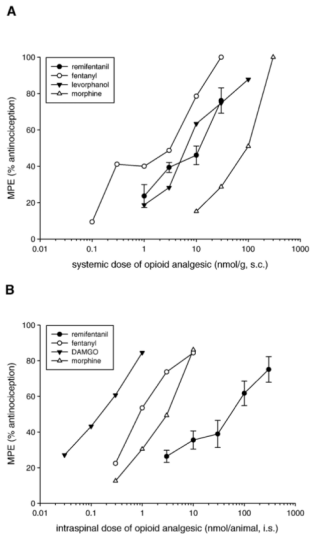

Fig. 3.

Relative antinociceptive potency of remifentanil following systemic (A) or spinal (B) administration in amphibians. Data points were plotted from the maximum peak effect during the 5-h time course as the mean peak effect (MPE +S.E.M.) of individual animals grouped for each dose. *N*=6–12 animals per dose. Data from previous studies was not plotted with error bars for clarity (from Stevens et al., 1994; Stevens, 1996). ED_{50} values, 95% confidence intervals, and relative potency of remifentanil and other opioids compared to morphine are given in Table 1.

Table 1

Antinociceptive potency of remifentanil after systemic and spinal administration in amphibians

Agent	Systemic administration		R.P.[b]	Spinal administration		R.P.
	ED_{50} (nmol/g)	(95% C.I.)[a]		ED_{50} (nmol/frog)	(95% C.I.)	
Remifentanil	7.1	(4.7–10.7)	12.2	3.2	(1.2–9.0)	0.7
Morphine	86.3	(64.7–115)	1.0	2.3	(1.7–3.0)	1.0
Fentanyl	1.4	(0.9–2.3)	61.6	0.9	(0.5–1.9)	2.4
Levorphanol	7.5	(5.1–11.1)	11.5	–		
DAMGO[c]	–[d]			0.1	(0.1–0.8)	17.4

[a] 95% confidence interval, ED_{50} values with non-overlapping intervals are statistically different at $P < 0.05$;

[b] relative potency compared to morphine (ED_{50} morphine/ED_{50} agent);

[c] [D-Ala^2,NMePhe^4,Gly-ol]-enkephalin;

[d] — (not tested). Morphine, fentanyl, levorphanol, DAMGO data from Stevens et al. (1994) and Stevens (1996).