Opioid-Induced Respiratory Depression and Analgesia May Be Mediated by Different Subreceptors

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Use of selective delta opioid antagonists provide evidence that the delta receptor within the brain seems an integrated part in the mediation of respiratory depression induced by a potent analgesic like fentanyl. Low doses of the delta antagonists RX-8008M (3–6 μg/kg) as well as ICI 174,864 (3–6 μg/kg) reversed fentanyl-related respiratory depression (arterial blood gases) in the unanesthetized canine. Opioid-induced blockade of afferent sensory nerve volleys (amplitude height of the somatosensory-evoked potential) could be reversed only by a high dose (9 μg/kg) of RX-8008M. Depression of amplitude height of the SEP could not be reversed by ICI 174,864 over the whole dose range (3–6–9 μg/kg). In comparison, naloxone (1–5–10 μg/kg) not only reversed depression of P_aO_2, it also reversed the blockade of afferent sensory nerve impulses in the low (5-μg/kg)-dose range. A highly selective delta antagonist may have a therapeutic value in reversing opioid-related respiratory depression, resulting in little or no attenuation of analgesia.

KEY WORDS: opioid receptors; fentanyl; RX-8008M; ICI-174,864; naloxone; somatosensory-evoked potential (SEP); respiration.

INTRODUCTION

Despite the discovery of multiple opioid receptors (1–3), it is the common notion that analgesia and respiratory depression present a functional entity of potent central analgesics which is related to the mu receptor (4). Using *in vivo* and *in vitro* experiments, however, it has been shown that the mu receptor functionally interconverts (5) or interacts through noncompetitive molecular mechanisms with the delta-binding site (6–8), which results in a potentiation of effects. The present study reports on a putative functional interaction of the mu-ligand fentanyl with the delta site, using the key elements of opioid effects, i.e., afferent sensory nerve blockade and respiratory depression and their competitive antagonism with delta antagonists of different selectivity.

MATERIALS AND METHODS

Mongrel dogs (n = 10), trained to lie unrestrained in the left lateral position and breathing room air, were given fentanyl 20 μg/kg i.v. over a period of 2 min. Five minutes later, the opioid antagonist RX-8008M was given in increasing doses (3–6–9 μg/kg i.v.) at 5-min intervals. This antagonist is a derivative of the oripavine series (16-methyl-cyprenorphine) which, compared to naloxone, has a 13-fold higher selectivity for the delta receptor (9) (Fig. 1).

On a separate occasion in 10 experiments, instead of RX-8008M, an even more delta-selective antagonist, however, of peptide nature (ICI-174,864; *N,N*-diallyl-Tyr-Aib-Aib-Phe-Leu-OH), was given in cumulative doses (3–6–9 μg/kg i.v.) after fentanyl at 5-min intervals. *In vitro* studies have demonstrated superior delta selectivity, making this compound an interesting candidate to unmask any delta receptor-mediated function (10). Also, 10 experiments were performed using cumulative doses of naloxone (1–5–10 μg/kg), the classical opioid antagonist, after fentanyl at similar time intervals. The doses of this antagonist were selected according to clinical use, which supports the notion of a low-dose reversal of postnarcotic respiratory depression.

In order to test whether redistribution and/or compensatory mechanisms account for the reversal of opioid effects, fentanyl was given on five separate occasions where, instead of an antagonist, only saline was administered in the same time sequence.

Before and after every drug injection the following parameters were measured:

(1) arterial blood gases (P_aO_2 and P_aCO_2) of samples drawn from an indwelling arterial catheter in the femoral artery to evaluate the degree of respiratory depression; and
(2) somatosensory-evoked potentials as derived from the sensory cortex (Ag/AgCl cup electrodes) positioned at Cz active, 2 cm rostral to the intraauricular plane on the animals scalp (10/20 system), to evaluate a functional deficit of afferent sensory nervous pathways.

Signals were induced via two stick-on electrodes by rectangular stimuli of electrical origin (0.2-msec duration, 1 mA above motor threshold, 5-Hz frequency) at the right front paw (Digi Stim II, Neuro Technology, Houston, Texas). A total of 256 stimuli was fed into a computer and averaged (Lifescan, Neurometrics, San Diego, Calif.). Peak-to-peak amplitude changes of the major deflection, centering around 50 msec poststimuli, were identified by cursor positioning. The height was automatically computed, and the results printed on paper by a built-in printer.

Previous studies in man (11–14) have demonstrated that the amplitude height of the evoked potential correlates closely with individual pain sensations. Thus, this method seemed to be a reliable and objective parameter to evaluate analgesia in the dog, as it had already been demonstrated by others (15–18).

Statistical Analysis

All data are given as mean ± SD. The peak-to-peak amplitude difference of the major deflection, centering aroung 50 msec poststimuli, was used to evaluate quantitatively the response to noxious stimuli. Thus, the amplitude height of the control period was compared with the peak changes after fentanyl, after increasing doses of ICI 174,864,

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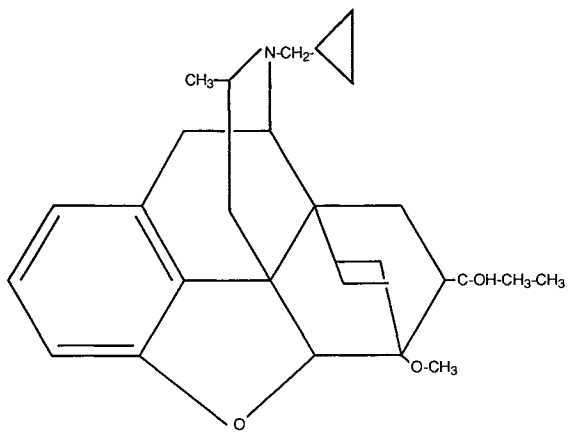

Fig. 1. The chemical structure of RX-8008M (16-methyl-cyprenorphine), a selective delta antagonist of the oripavine series.

RX-8008M, saline, and naloxone, respectively. Also, the arterial blood gases (P_aO_2 and P_aCO_2), taken 5 min after the different medications, were compared with the control period. The Wilcoxon, Mann, Whitney test for paired observation with Bonferroni correction was used for statistical analysis. $P \leq 0.05$ was considered significant.

RESULTS

Compared to the awake-control situation, fentanyl induced a depression of respiration 5 min after the injection. This was reflected in a drop of P_aO_2 from a mean of 98 to a mean of 65 Torr ($P < 0.01$) and a rise of P_aCO_2 from 35 to a mean of 47 Torr (Table I). Five minutes after 3 μg/kg of the antagonist RX-8008M, P_aO_2 increased to a mean of 98.9 Torr and P_aCO_2 was reversed to a mean of 37 Torr. These values were not significant compared to the control. The second dose of RX-8008M (6 μg/kg) further increased P_aO_2 to 102 Torr and the third dose (9 μg/kg) to a mean of 103 Torr (Table I).

Fentanyl also had a significant effect on the amplitude height of somatosensory-evoked potentials (SEPs). Compared to the control period the opioid induced a depression ($P < 0.01$) of amplitude height of the evoked potential (N_{50}-peak) from a mean of 6.9 to a mean of 0.98 μV (Table I). The depression was accompanied by no defense reaction to clamping of the tail. Only the second dose of RX-8008M (6 μg/kg) increased the amplitude to a mean of 3.6 μV. Depression, however, was still significant ($P < 0.05$) compared to the control (Table I). The third dose (9 μg/kg) resulted in an overshoot reaction to a mean of 9.3 μV, surpassing control values (Table I). The overshoot, however, was not significant compared to the control. The increase in amplitude height of the SEP was accompanied by marked defense reaction to clamping of the tail.

The peptide antagonist ICI 174,864 also reversed hypoxia as a result of fentanyl ingestion. P_aO_2 increased from a mean of 66 to 87, 94, and 102 Torr, respectively, following incremental doses of 3, 6, and 9 μg/kg. The delta antagonist ICI 174,864, however, did not reverse fentanyl-related amplitude depression of the evoked potential (Table I). Mean amplitude height of the SEP had dropped from a mean of 7.1

Table I. The Effects of the Opioid Fentanyl on Arterial Blood Gases (P_aO_2, P_aCO_2 in Torr) and on the N_{50} Peak of the SEP (μV) Followed by Various Antagonists and by Saline, Respectively (Mean ± SD)

	Saline group				
	Control	Opioid	Post 5 min	10 min	20 min
P_aO_2	94.8 ± 6.9	58.7 ± 7.6	65.9 ± 0.6	70.9 ± 3.2	75 ± 6.2
P_aCO_2	32.3 ± 2.7	51.3 ± 2.2	47.7 ± 2.3	38.0 ± 4.4	35 ± 5.4
SEP	7.7 ± 3.2	1.3 ± 0.8	1.4 ± 1.3	1.5 ± 0.9	1.9 ± 1.0
	RX-8008M group				
	Control	Opioid	3 μg/kg	6 μg/kg	9 μg/kg
P_aO_2	98 ± 1.0	65 ± 5.0	98.9 ± 7.0	102 ± 2	103 ± 1.9
P_aCO_2	35 ± 2.0	47 ± 4.0	37.0 ± 3.0	35.0 ± 3.5	33.0 ± 2.5
SEP	6.9 ± 2.2	0.98 ± 0.5	2.2 ± 1.7	3.6 ± 1.1	9.3 ± 0.9
	ICI 174,864 group				
	Control	Opioid	3 μg/kg	6 μg/kg	9 μg/kg
P_aO_2	94.9 ± 9.0	66 ± 6.3	87 ± 5.5	94 ± 6.5	102 ± 7.7
P_aCO_2	34 ± 2.7	47 ± 3.9	39 ± 4.9	38 ± 5.0	34 ± 4.0
SEP	7.1 ± 2.0	1.1 ± 0.9	0.98 ± 0.5	1.0 ± 0.9	1.1 ± 0.7
	Naloxone group				
	Control	Opioid	1 μg/kg	5 μg/kg	10 μg/kg
P_aO_2	94.8 ± 6.9	59.7 ± 7.6	68.9 ± 6.0	92.7 ± 9.9	100 ± 2.6
P_aCO_2	34 ± 2.5	49.9 ± 3.0	45.0 ± 5.0	33.8 ± 2.0	32.7 ± 2.5
SEP	7.8 ± 2.4	1.3 ± 0.8	1.5 ± 1.7	7.4 ± 1.1	9.0 ± 0.9

to 1.1 μV ($P < 0.01$) after fentanyl injection. Following increasing doses of ICI-174,864 amplitude height remained around a mean value of 1.0 μV, which is significant ($P < 0.01$) compared to the control (Table I).

Fentanyl-related effects, i.e., respiratory depression and amplitude reduction of the SEP, were reversed by naloxone. While 1 μg/kg had little effect, there was a reversal of respiratory depression (P_aO_2 increased from 68.9 to 92.7 Torr) and an increase in amplitude of the SEP from 1.5 to 7.4 μV after 5 μg/kg (Table I). The third dose of the antagonist (10 μg/kg) resulted in a further improvement of partial arterial oxygen pressure to a mean of 100 Torr and a facilitation of afferent sensory nervous volleys, resulting in an increase in amplitude to a mean of 9.0 μV (Table I).

The low partial oxygen pressure of those animals receiving saline after fentanyl showed some recovery. However, there was no full recovery in the 20 min following fentanyl and there was statistical significance ($P < 0.05$) between the saline, the RX-8008M, ICI 174,864, and the naloxone group (Table I).

DISCUSSION

Although the existence of separate opioid receptors in the brain (mu, kappa, delta) is now established, their physiological significance is only slowly being evaluated. It is generally accepted that the mu receptor at the supraspinal level is involved in the mediation of analgesia (19,20). Respiratory depression allegedly is linked to the mu and/or the delta receptor interaction (21,22). Using selective *antagonists* with different specificity for the two receptor sites (mu and delta), it was possible to demonstrate some separation of analgesia and respiratory depression.

The antagonist, RX-8008M, which has a 16 times higher delta selectivity than the commonly used antagonist naloxone (9), reversed fentanyl-induced respiratory depression with little impairment of nociceptive blockade in the lower dose range. Higher doses not only induced a complete recovery in the propagation of nociceptive impulses but resulted in an overshoot reaction also seen after naloxone reversal. The latter was derived from SEP measurements and the response to tail clamping, suggesting a dominance of mu-receptor interaction at high doses of this antagonist. The results suggest that analgesia depends primarily on mu-receptor occupation and is underlined by the ease of reversal of the depressed amplitude of the SEP with naloxone, an antagonist with preferential affinity for the mu receptor (23).

The peptide antagonist ICI 174,864, which in isolated tissue preparations, has a higher selectivity to the delta site than any other compound (10), also reversed fentanyl-induced respiratory depression. The response to an electrical stimulus, which measured the neural events in pain perception and process (24), was not reversed over a wide dose range. Since it was demonstrated that recovery from opioid-induced respiratory impairment and sensory blockade is not related to the sole distribution of the opioid (saline group), the present data favor the hypothesis of different receptors being functionally involved in the mediation of opioid effects: one site which preferentially mediates mu-related analgesia and another site, the delta-binding site, which is involved in the mediation of respiratory depression. The latter assumption is underlined by the observation that endogenous opioids, which preferentially bind to the delta receptor, are found at high concentrations in the brain stem of vertebrate animals and man. Delta-binding sites thus seem to play an important part in the central control of breathing (25). The peptide antagonist at 3 μg/kg, compared to a similar dose of RX-8008M, was less effective in reversing opioid-related hypoxemia. This may be due to the commonly known poorer penetration of peptides through the blood–brain barrier of the CNS, which may account for an insufficient occupation of the ligand to the receptor site(s) involved in the mediation of respiratory effects.

Mu isoreceptors (mu_1 and mu_2), on the other hand, could be responsible for the mediation of analgesia and respiratory depression, respectively (21,26). This assumption is based on data with a long-lasting noncompetitive antagonist, i.e., naloxonazine, which allegedly interacts with the mu_1 subreceptor, reversing analgesia without affecting respiratory depression. However, mu_1 selectivity of naloxonazine has not been demonstrated sufficiently. Since naloxonazine is a noncompetitive mu_1 antagonist, reversal of effects becomes apparent only *24 hr* after administration (21). By this time an irreversible conformational change of the mu as well as the delta receptor may have very likely emerged. This notion is underlined by data where naloxonazine, similar to ICI-174,864, reversed delta receptor-mediated urinary bladder contraction, suggesting simultaneous delta-receptor interaction (27).

Based on the present results and the data of other researchers (7), an extended model of receptor interaction is put forward. Aside from the purported potentiation of analgesia by simultaneous delta-receptor interaction (8), the common respiratory depression of potent mu ligands is mediated via simultaneous delta interaction. Delta binding of opioids not only enhances analgesia but brings about respiratory impairment via a functional interaction. Such a close functional mu/delta interaction is supported by the following.

(1) Assuming mu receptor mediation of analgesia, there is an obvious lack between mu receptor affinity and analgesic potency, and certain opioid peptides can either increase or even diminish morphine-induced analgesia (8).
(2) Compounds such as fentanyl and sufentanil may owe their great potency to the simultaneous binding to the mu and the delta receptor. Their morphine part confers mu activity, while their phenylethyl substituent interacts with the delta-binding site (3).
(3) Morphine, β-endorphin, and D-ala-2-met-5-enkephalinamid induce a similar cessation of respiratory drive in neonatal and adult rats. In the adult animal, however, morphine analgesia occurs at 40-fold lower doses, which is due to an increase in mu binding during maturation (28).
(4) The highly selective mu antagonist β-FNA, at doses which produced morphine antagonism of antinociception, does not alter opioid-induced depression of respiratory rate (29).

(5) Occupancy of the mu receptor with β-FNA prevented the usual therapeutic response of the delta antagonist ICI 154,129 in endotoxemic rats (6).

In conclusion, the above experiments put forward strong evidence that, aside from mu binding, simultaneous delta-interaction of fentanyl is important for the expression of opioid-induced respiratory impairment. An antagonist with high selectivity for the delta site could be a potentially interesting candidate for the reversal of respiratory depression, retaining analgesia after opioid administration. It remains to be seen, however, whether similar results can be obtained in man.

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