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The Effects of Intravenous Naltrindole and Naltrindole 5′-Isothiocyanate on Sufentanil-Induced Respiratory Depression and Antinociception in Rats

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VERBORGH, C. AND T. F. MEERT. *The effects of intravenous naltrindole and naltrindole 5′-isothiocyanate on sufentanil-induced respiratory depression and antinociception in rats.* PHARMACOL BIOCHEM BEHAV **63**(1) 175–183. 1999.—Although the interactions between the μ- and the δ-opiate receptor subtypes are well documented with regard to supraspinal analgesia, less is known about the mutual interactions on respiratory depression. To clarify the functional interactions between both opiate receptor subtypes with regard to antinociception and respiratory depression, male Wistar rats were intravenously injected with 2.5 μg/kg of the μ-opiate agonist sufentanil and subsequently intravenously challenged with the delta antagonist naltrindole (NTI) or naltrindole 5′-isothiocyanate (5′-NTII), a δ-2 antagonist. Antinociception was measured by means of the tail-flick latency, and respiratory depression was evaluated by means of analysis of $PaCO_2$, PaO_2, and oxygen saturation. To quantify the antagonistic properties of NTI and 5′-NTII, mean areas under the curve were calculated for groups treated with sufentanil, control vehicle, and sufentanil plus a dose of the antagonists. NTI, but not 5′-NTII, antagonized the sufentanil-induced antinociception at 10 mg/kg NTI. Below this dose the effects were inconsistent. The sufentanil-induced hypercapnia and hypoxia were diminished with 10 mg/kg NTI or 5′-NTII. These data indicate that NTI antagonizes the sufentanil-induced antinociception and respiratory depression in rats. A dissociation between the antinociception and respiratory depression following intravenous sufentanil could be obtained with 10 mg/kg 5′-NTII pointing to different regulatory effects of opiate δ receptor subtypes on μ-opiate agonist-induced behavioral effects. © 1999 Elsevier Science Inc.

Sufentanil Naltrindole Naltrinole 5′-isothiocyanate Antinociception Respiratory depression

EXPERIMENTS performed in the late seventies revealed a difference in potency of enkephalins in a guinea pig ileum and a mouse vas deferens assay, leading to the postulation of multiple opiate receptors in these assays, including the delta (δ)-opiate receptors (27). Subsequent evidence demonstrated the involvement of δ-opiate receptors in antinociceptive processes. Several articles documented the contribution of δ-opiate receptors in the analgesic potency of metkephamid, an analog of Met-enkephalin (13,20). The functional role of μ and δ opiate receptors was controversial with regard to supraspinal analgesia, and several types of interactions between both opiate receptor subtypes with regard to μ-opiate–mediated antinociception were reported (3,17,18,60). However, no firm conclusion has been drawn yet concerning the functional interaction of both opiate systems in the modulation of antinociception.

With regard to respiratory depression, the crossinteractions between the μ and δ opiate receptors are even less well understood, and only a few articles describe the role of the δ opiate system on respiratory depression induced by a μ-opiate receptor agonist (14,24). Although it is generally accepted that peptides acting as δ-opiate receptor agonists induce anti-

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nociception when given intracisternally, intracerebroventricular, or systemically, the literature on the respiratory effects of these peptides is very conflicting. For instance, several articles report respiratory depression following microinjections of δ agonists in rat brain nuclei (15,41,42) or following intracerebroventricular injections in dogs (19,40,50). Contrary to this, several other articles fail to demonstrate a decrease in respiration after systemic, intrathecal, or even intracerebroventricular administration of δ opioids (11,26,30,38).

Recently, two pharmacologically distinct δ-opiate receptor subtypes were described on the basis of a series of behavioral and biochemical studies (22,29,37,53). Various selective agonists and antagonists became available. Cyclic[D-Pen^2, D-Pen^5]enkephalin (DPDPE) and 7-benzylidenenaltrexone (BNTX) were characterized as a selective δ-1 agonist (36) and a δ-1 antagonist (44,52), respectively. For the δ-2 opiate receptors, [D-Ala^2 Glu^4]deltorphin, was described as a selective and high-affinity agonist, originally derived from a frog skin extract (10). Naltrindole 5′-isothiocyanate (5′-NTII) was proposed as a nonequilibrium δ-2 opiate receptor antagonist (37,45). We studied the ability of δ-opiate receptor antagonists to selectively modulate respiratory depression and antinociception following a μ-agonist as a result of a number of observations by others. First, some δ-opiate receptor agonists were demonstrated to potentiate the antinociceptive properties of morphine while reducing some side effects (21). Second, morphine dependence and tolerance can be prevented by δ-opiate receptor antagonists (1,34); and third, morphine antinociceptive tolerance and tolerance to the respiratory depression of morphine were differentially blocked by naltrindole (16). Initial observations in rat brain membranes also showed that [D-Ala^2,D-Leu^5]-enkephalin (DADLE) binds to the high affinity or μ-1 opiate receptor site with an almost equal affinity as [D-Ala^2, MePhe^4, Gly-ol^5]-enkephalin (DAMGO) (25,28).

We wondered whether the antinociception and respiratory depression induced by an intravenously administered μ-opiate receptor agonist such as sufentanil, affecting both the high- and the low-affinity μ-opiate receptor sites could be differentially blocked by naltrindole (NTI) or naltrindole 5′-isothiocyanate (5′-NTII), two nonpeptide and more stable δ-opiate receptor antagonists (46). Although some reports suggest that supraspinal analgesia may be predominantly mediated by δ-2 subtype receptor occupation (39,48), others propose involvement of both subtypes (57). Other effects, however, which are possibly centrally regulated, like hypoxic adaptation in mice (31) or opioid induced muscle rigidity in rats (59), are mediated by the δ-1 receptor. Although no data are available that indicate the involvement of δ-1 receptors in the respiratory drive, there is one report describing both subtypes of δ receptors in anatomical locations contributing to respiration (57). The physiologic effects resulting from a receptor block sparing the δ-1 subtype receptor may shed some light on this aspect of respiration. The selection of a specific δ-2 opiate receptor antagonist was further spurred by recent reports on the δ-2 opiate receptor involvement in modulation of supraspinal morphine-induced antinociception (22,43), morphine-induced dependence (33,35,37) and stress-induced antinociception (58).

METHOD

Animal Preparation

Approval from the Institutional Animal Care and Use Committee was obtained to perform the experiments described. Seventy-seven naive male Wistar rats, weighing 215–310 g, were housed in a room with controlled temperature (21 ± 1°C) and humidity (65 ± 5%) and a light–dark cycle (lights on 0700–1900 h). Food and water were provided ad lib. On the day of the experiment, the animals were anesthetized with 0.4 mg/kg intraperitoneal etomidate. After preparation of the left femoral artery, a polyethylene catheter (PE 50) was inserted into the proximal end for 3 mm. The rats were placed in Bolman cages to emerge from anesthesia. A Bolman cage is a Perspan construction holding eight adjustable longitudinal metal rods arranged as a cylinder, allowing liberal movement of head, paws, and tail, and also permitting to a certain extent abdominal and thoracic movement. The rods of the cages were adjusted as to minimize impairment of respiratory movements, as any obstruction to normal thoracic or abdominal expansion would seriously alter blood gas variables. The animals were kept in these Bolman cages during the entire observation period. Before and during the experiments the femoral lines were kept patent using 200 μl injections of a solution of Heparin sodium (100 U/ml). All observations and blood sampling were done between 1000 and 1200 h.

Chemicals

Sufentanil citrate (Janssen Pharmaceutica, Beerse, Belgium), naltrindole hydrochloride, and naltrindole 5′-isothiocyanate (R.B.I., Natick, MA) were dissolved in saline in different recipients in such a way that injection of a certain dose always consisted of 250 μl. Intravenous injections were given in a volume of 0.2 ml per 100 g body weight.

Assessment of Respiratory Function

Arterial blood samples (200–250 μl) were collected from the femoral cannula into 1-ml heparinized syringes. Blood samples were kept on ice until analysis. The pH, paO_2, $paCO_2$, oxygen saturation, bicarbonate, and base excess were determined using a blood gas analyzer (ABL3 Radiometer Copenhagen®).

Assessment of Antinociception

The time to withdrawal of the tail (tail-flick latency: TFL) being immersed for 5 cm into a bath filled with demineralized water kept constant at 55 ± 1°C (Julabo Labotechnik®) was recorded to the nearest 0.1 s. To minimize tissue damage on repetitive measurements a cutoff time of 10.0 s was used. A TFL >6.0 s never occurred in control animals.

Experimental Design

At least 3 h elapsed to permit recovery from etomidate anesthesia. Recovery was confirmed by a TFL <2 s, normal behavior, recovery of muscle tone, and normalization of the blood gas values. The rats then received either 2.5 μg/kg sufentanil (n = 66) or vehicle control (n = 11) injected via a lateral tail vein. Aspiration of blood confirmed intravenous location of the needle. The antagonist was injected intravenously 5 min after opioid administration. Naltrindole and naltrindole 5′-isothiocyanate were both given at concentrations of 0.16, 0.63, 2.5, and 10 mg/kg (n = 7 rats per dose). Tail flick latencies and blood gases were assessed before the sufentanil injection, at 5 min after sufentanil but before the administration of an antagonist, and subsequently at 2, 5, 10, 15, 20, 30, 45, and 60 min following administration of the opioid antagonist. Tail flick latencies were measured after blood sampling

to avoid the influence of the thermal stimulation on the blood gas values.

Data Analysis

The percent inhibition compared to the mean values of both the sufentanil and the vehicle controls at time *t* was calculated according to the formula:

$$\% \text{ inhibition } (t) = [\text{mean TFL}(t) \text{ (sufentanil series)} - \text{TFL } (t)] \times 100/[\text{mean TFL}(t) \text{ (sufentanil series)} - \text{mean TFL}(t) \text{ (vehicle series)}].$$

where TFL (*t*) is the tail flick latency measured at time *t*; mean TFL (*t*) (sufentanil series) is the mean tail-flick latency measured at time *t* in rats treated with sufentanil alone; mean TFL (*t*) (vehicle series) is the mean tail-flick latency measured at time *t* in rats treated with only vehicle.

Respiratory variables were calculated accordingly.

Areas under the curve (AUC) were calculated as the difference between the area constituting the time course of a particular variable following sufentanil injection without any antagonist and those of sufentanil plus an antagonist. Areas were calculated for an observation period of 60 min. Statistical analysis included a two-factor ANOVA, while the Mann–Whitney *U*-test (two tailed) was further used for the evaluation of differences between the different groups. Differences with respect to preinjection values were tested with the Wilcoxon test (two tailed).

RESULTS

Effects on Antinociception

An intravenous injection of 2.5 μg/kg sufentanil increased the TFL to more than 10.0 s in all sufentanil-treated animals for at least 20 min. The TFL was more than 6.0 s in all sufentanil-treated animals during the entire observation period. A significant difference ($p < 0.05$) in the TFL after sufentanil followed by an antagonist compared to sufentanil alone was obtained with 0.63, 2.5, and 10 mg/kg naltrindole (Fig. 1) at respectively 15, 10, and 5 min after the administration of the antagonist; the difference lasted for the whole observation period with 10 mg/kg naltrindole. Only 10 mg/kg naltrindole completely antagonized the TFL to baseline levels obtained with the vehicle controls. This effect was only observed after some time. Naltrindole 5′-isothiocyanate only slightly affected the mean TFL values; at no time was a significant reduction observed. The mean TFL values of naltrindole were mostly significant lower than the comparable values of naltrindole 5′-isothiocyanate.

The AUCs for the TFL after sufentanil with an antagonist were significantly different from sufentanil alone after 0.63 and 10 mg/kg naltrindole (Fig. 2). Only at 10 mg/kg naltrin-

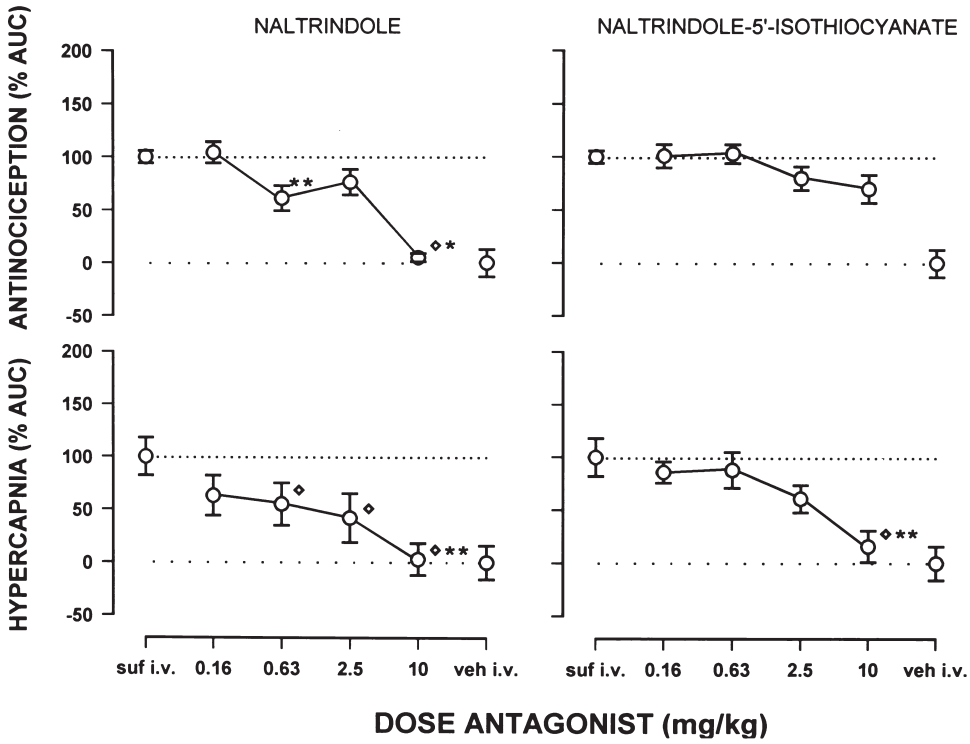

FIG. 1. Time course of the mean tail-flick latency (TFL) following 2.5 μg/kg intravenous sufentanil without antagonist (closed squares) ($n = 10$) or 2.5 μg/kg sufentanil followed by an intravenous administration with 0.16 mg/kg (open circles), 0.63 mg/kg (open triangles), 2.5 mg/kg (open diamonds), or 10 mg/kg (open semicircles) naltrindole (lower panel) or naltrindole 5′-isothiocyanate (upper panel). The number of rats in each antagonist treated group was 7. The mean TFL in rats given twice vehicle (controls; ctrl; $n = 11$) are shown as open squares. The standard error of the mean (SEM) controls is lower than 0.19 s, while SEM in decreasing tail flick latencies was 0.11–1.25 s.

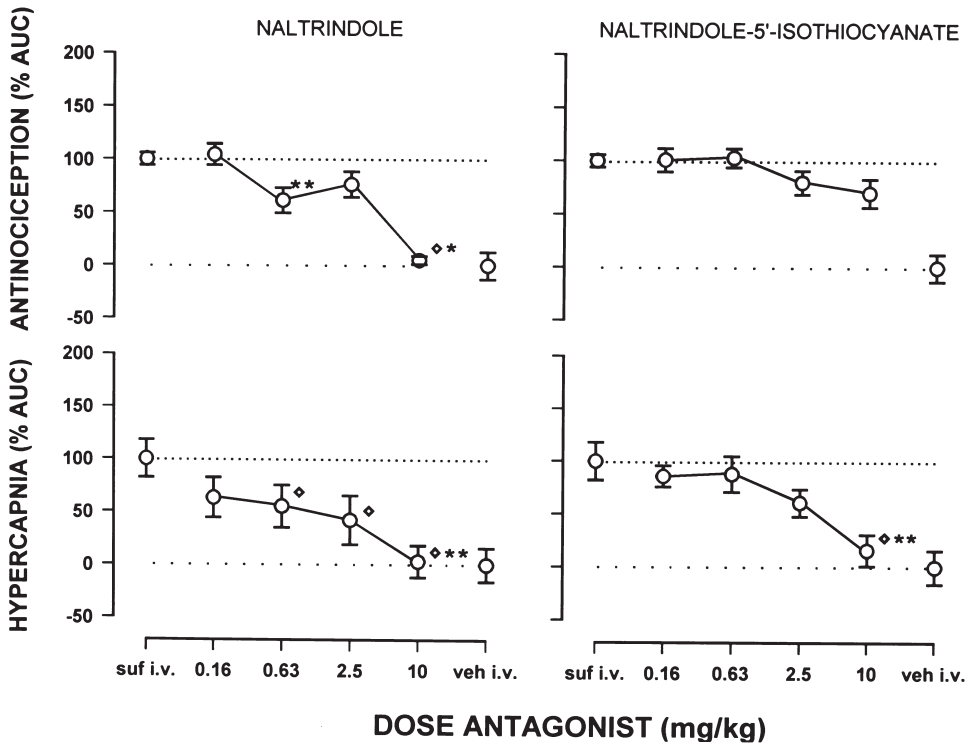

FIG. 2. Area under curve (AUC) of the time course of the TFL (top panels) and the $PaCO_2$ (bottom panels) obtained after a 60-min observation period. The areas were calculated as percentages of the area obtained in rats given sufentanil ($n = 10$) (100% area). The area in rats given vehicle was taken as reference (controls; ctrl) ($n = 11$) (0% area). Shown are the areas following different doses of naltrindole (left panels) and naltrindole 5′isothiocyanate (right panels) administered intravenously at 5 min following sufentanil. An open diamond (◇) indicates that the mean areas are not different from those of the vehicle controls ($p < 0.05$; two tailed). Differences with sufentanil were evaluated using the Mann–Whitney *U*-test (two tailed: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$).

dole, the AUCs for the TFL over a period of 60 min did not differ from those of the nonsufentanil-treated controls.

Effects on Hypercapnia

The injection of 2.5 μg/kg sufentanil immediately increased $PaCO_2$ values by at least 10%, and gradually returned to baseline by 45 min (Fig. 3). Antagonist-treated animals had $PaCO_2$ levels that returned to baseline more rapidly than animals given sufentanil alone at any dose. Baseline was reached after 5 min for 10 mg/kg naltrindole 5′-isothiocyanate and after 30 min for 10 mg/kg naltrindole. The 10 mg/kg dose of either antagonists reduced the hypercapnia 2 min after administration. The 10 mg/kg dose was also the concentration at which significantly lower $PaCO_2$ levels were measured in the antagonist groups compared to the plain sufentanil group. $PaCO_2$ values not different from those of vehicle-treated animals were found after 2.5 and 10 mg/kg naltrindole and at 10 mg/kg naltrindole 5′-isothiocyanate. The larger the dose, the sooner the antagonist returned the $PaCO_2$ values back to the control values measured in the vehicle-treated control animals. There were no differences in the mean $PaCO_2$ levels between comparable doses of both antagonists.

In terms of the AUC, the sufentanil-induced hypercapnia was antagonized with 10 mg/kg naltrindole or naltrindole 5′-isothiocyanate to mean levels not different from the vehicle controls (Fig. 2). The AUC over the 60-min observation period also indicated that at 0.63 and 2.5 mg/kg naltrindole, the mean $PaCO_2$ levels were not different from those of the vehicle controls.

Effects on Hypoxia and Oxygen Saturation

Arterial oxygen tension decreased by at least 20% following an intravenous administration of 2.5 μg/kg sufentanil. The decrease in PaO_2 was significant during nearly 20 min, and the decrease in oxygen remained significant for 45 min. The mean PaO_2 levels returned to presufentanil levels within 2 min following 10 mg/kg naltrindole and within 10 min following 10 mg/kg naltrindole 5′-isothiocyanate. Compared to sufentanil-treated animals, these doses significantly increased the PaO_2 and the oxygen saturation during the entire observation period. The mean PaO_2 values obtained with these doses of the antagonists were comparable to the control values from 15 and 20 min after administration. For the oxygen saturation, a return to vehicle control baseline values were measured at 2 min after 10 mg/kg of both antagonists, and after 10 min following the 2.5 mg/kg dose of both antagonists. The administration of a dose of 10 mg/kg of both antagonists resulted in significantly different AUCs for the PaO_2 and the oxygen saturation compared to the sufentanil-treated animals (Table 1). At the dose of 10 mg/kg of both antagonists, the AUCs for the

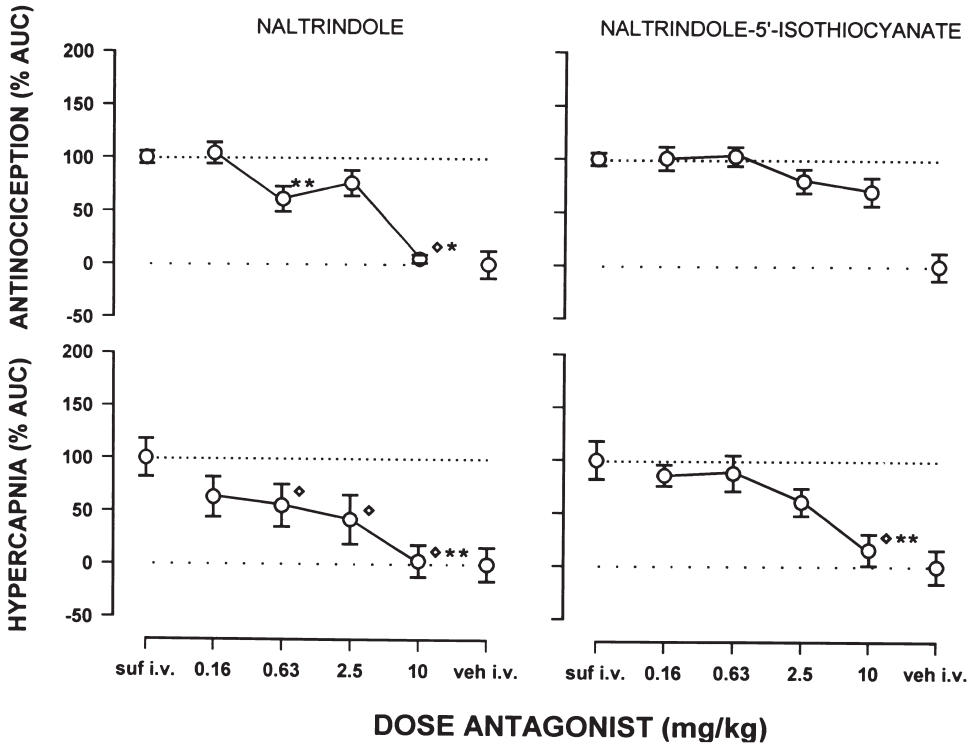

FIG. 3. Time course of the mean arterial carbon dioxide level $PaCO_2$ in mmHg following an intravenous administration of 2.5 μg/kg sufentanil without antagonist (closed squares) or with 0.16 mg/kg (open circles), 0.63 mg/kg (open triangles), 2.5 mg/kg (open diamonds), or 10 mg/kg (open semicircles) naltrindole (lower panel) or naltrindole 5′-isothiocyanate (upper panel). The antagonists were given intravenously at 5 min after sufentanil. $PaCO_2$ in rats given twice vehicle (controls; ctrl) is shown as open squares. SEM of the displayed symbols varied between 0.46 and 2.80 mmHg. See also legend of Fig. 1 for the number of rats in each group.

PaO_2 and oxygen saturation returned to the baseline control values. The 2.5 mg/kg dose of either antagonist was able to antagonize the drop in oxygen saturation provoked by sufentanil, as evidenced by an AUC of the oxygen saturation not different from vehicle control (Table 1). The comparison of the respective doses of naltrindole and naltrindole 5′-isothiocyanate revealed no differences in antagonizing the decrease in PaO_2 or oxygen saturation following sufentanil.

Effects on pH

The pH of the blood taken in animals receiving only sufentanil decreased from 7.36 (0.03) to 7.24 (0.13). The pH measured in the blood of rats treated with vehicle, sufentanil, or sufentanil followed by one of the antagonists varied minimally, and was not significantly different among or between the different treatment groups (Table 2).

DISCUSSION

The aim of the present study was to investigate the role of two δ-opiate receptor antagonists on the intravenous sufentanil-induced antinociception and respiratory depression. The results of the experiments indicate that an intravenously administered dose of 10 mg/kg naltrindole 5′-isothiocyanate can reverse the sufentanil-induced respiratory depression, without affecting the antinociceptive activity. With naltrindole, an antagonism of both sufentanil-induced effects was observed. The doses of the antagonists used represent almost equimolar concentrations. Based on the presumed selectivity of naltrindole 5′-isothiocyanate for δ-2 opiate receptor subtypes, these results suggest that either naltrindole is capable of displacing sufentanil from the μ-receptors or that an additional δ-1 opiate receptor occupancy of sufentanil is a necessary condition to fully block the pain transmission and, as such, that δ-1 opiate receptor occupation by the antagonist naltrindole modulates the nociception. Thus, in other words, the δ-1 opiate receptors mediate, at least in part, intravenous opioid-mediated antinociception in rats.

The hypothesis that supraspinal antinociception can be mediated in part by δ-1 opiate receptor occupancy in rats is confirmed by a number of observations. In most of the studies looking at supraspinal antinociception in rats, the δ-1 opiate receptor agonist DPDPE was injected intracerebroventricularly, and appeared to have some efficacy in a number of nociceptive assays including the hot-plate test, the formalin test, a hot and a cold tail-flick test, and a measurement of mechanical nociceptive thresholds (6–8,32,51,54). The antinociceptive effect was usually obtained with a dose of 30 μg/rat. However, with doses up to 200 μg/rat in another study (3), no complete antinociceptive effect could be obtained, pointing to a limited impact of this δ-opiate system in the pain modulation. Furthermore, in studies where DPDPE was microinjected in the

TABLE 1
PERCENT DECREASE IN OXYGEN PARAMETERS

	paO_2		Saturation	
Antagonist	NTI	5′-NTII	NTI	5′-NTII
Sufentanil	100 (17)		100 (20)	
0.16 mg/kg	86 (20)	95 (17)	76 (24)	99 (20)
0.63 mg/kg	113 (12)	117 (18)	60 (22)	104 (18)
2.5 mg/kg	76 (17)	67 (12)	19 (21)◊*	45(29)◊
10 mg/kg	9 (19)◊†	21 (14)◊†	−7 (15)◊†	2 (19)◊†
Vehicle	0 (13)		0 (16)	

This table lists the decreases in PaO_2 and oxygen saturation observed in the blood gasses of rats treated with vehicle or sufentanil or treated with sufetanil followed by naltrindole (NTI) or naltrindole 5′-isothiocyanate (5′-NTII), both given IV. The data are expressed as percentages of the area under curve (AUC) of the time course of the PaO_2 or oxygen saturation obtained during 60-min observation of rats treated with sufentanil alone (=100% area). Mean values (SEM) are shown. The areas were calculated using the means recorded in rats given sufentanil and rats given vehicle as reference (0% area). Shown are the percentages area following different doses of naltrindole and naltrindole 5′-isothiocyanate administered intravenously at 5 min following sufentanil. An open diamond (◊) indicates that the mean areas are not different from those of the vehicle controls (Mann–Whitney *U*-test; $p < 0.05$; two tailed). Differences with sufentanil were evaluated using the Mann–Whitney *U*-test (two tailed: $*p < 0.05$ and $\dagger p < 0.01$).

periaquaeductal gray, locus coeruleus (5,39,49) or rostral ventral medulla (49), no analgesic response was observed. Also, injections in the right ventricle were reported to produce less than 50% of a maximal possible effect in the hot-plate test and a lack of activity in the tail-flick test (39). These observations, however, do not exclude the possible presence of DPDPE-sensitive receptors in pain-mediating structures different from these nuclei.

Further evidences for the role of δ-1 and not δ-2 opiate receptors in supraspinal antinociception in rodents came from observations in Swiss–Webster mice, demonstrating that the δ-1 opiate receptor agonist DPDPE, when coadministered with low doses of intracerebroventricular-injected morphine, significantly increased antinociception, while the δ-2 opiate receptor agonist [D-Ala^2]deltorphin II was without any effect (53). The increased antinociception with DPDPE was antagonized by the δ-opiate receptor antagonist naltrindole (NTI) and the δ-1 opiate receptor antagonist 7-benzylidenenaltrexone (BNTX), but not by the δ-2 opiate receptor antagonist naltriben (NTB) (55). The heroin-induced antinociception is also partly δ-1 opiate receptor mediated, as evidenced by an antagonism of these effects with the δ-1 selective opiate receptor antagonist BNTX (47). The δ-2 opiate receptors are probably not involved in supraspinal analgesia, as demonstrated in a study where naltriben did not change the antinociception induced by intracerebroventricular administration of DADLE, morphine sulfate, DAMGO, or U-50,488H (53).

In this study, the sufentanil-induced antinociception was reversed by the δ antagonist naltrindole. Although the bulk of literature complies with the classical high selectivity for μ or δ receptors, this is not the first study to report a reversal of the antinociception of a μ-opiate receptor agonist with a specific δ-opiate receptor antagonist. It was reported that 20 mg/kg naltrindole could partially block the analgesic activity of a fentanyl-related opioid to nearly 50% in CD-1 mice (4). As in this mouse study, high doses of naltrindole were also needed in our study to obtain an antagonism of the opioid-induced antinociception. An antagonism was only observed at the highest doses of naltrindole tested. Lower doses of the antagonist may be ineffective, as observed in a study where doses of 1–10 μg naltrindole were unable to block the antinociception induced by intracerebroventricular morphine (2). Microinjection studies also showed that antinociception following direct application of morphine in the mesencephalic area was significantly reduced by application of naltrindole (0.5 to 5 μg) to the rostroventral medulla in rats (23). Further, the antagonism of morphine induced antinociception can also be observed by coadministration of [Met^5]enkephalin, a phenomenon currently called negative modulation (43). However, the possibility of a loss of δ selectivity of the antagonists used in some of these studies cannot be excluded.

The fact that both naltrindole and naltrindole 5′-isothiocyanate reversed the sufentanil-induced respiratory depression, suggests that δ-2 receptors could be involved in the modulation of the sufentanil-induced respiratory depression. Only one article described the reversal of the intravenous sufentanil-induced respiratory depression following intravenous naltrindole (14). In this study in mongrel dogs, doses from 40–160 μg/kg naltrindole or naltriben were effective in antagonizing the sufentanil-induced hypercapnia and hypoxia, as evidenced

TABLE 2
EFFECTS ON pH VALUES

	NTI			5′-NTII		
Antagonist	Control	Sufentanil	10 min	Control	Sufentanil	10 min
Sufentanil	7.36 (0.03)	7.24 (0.13)	7.25 (0.14)			
0.16 mg/kg	7.36 (0.04)	7.26 (0.06)	7.24 (0.07)	7.34 (0.02)	7.28 (0.05)	7.28 (0.04)
0.63 mg/kg	7.38 (0.03)	7.26 (0.03)	7.29 (0.03)	7.37 (0.03)	7.27 (0.03)	7.25 (0.07)
2.5 mg/kg	7.34 (0.05)	7.25 (0.04)	7.27 (0.06)	7.35 (0.02)	7.24 (0.02)	7.27 (0.04)
10 mg/kg	7.35 (0.03)	7.24 (0.01)	7.31 (0.05)	7.35 (0.03)	7.25 (0.02)	7.3 (0.05)
Vehicle	7.37 (0.05)	7.36 (0.08)	7.36 (0.14)			

This table represents the pH values measured in the blood gases of rats treated with vehicle or sufentanil or treated with sufentanil followed by naltrindole (NTI) or naltrindole 5′-isothiocyanate (5′-NTII), both given IV. Mean values (SEM) are shown. The data shown here are from samples taken before intravenous sufentanil injection (control), shortly after the sufentanil, but before administration of the antagonist (sufentanil) and 10 min following injection of either naltrindole (NTI) or naltrindole 5′-isothiocyanate (5′-NTII) at 0.16, 0.63, 2.5, and 10 mg/kg (10 min). Differences among or between groups are not significant.

by blood gas analyses. Somatosensory evoked potentials were used to evaluate antinociception. The decrease in averaged recorded voltages provoked by sufentanil was equally reversed by naltrindole and naltriben. But naltriben reversed the changes in blood gas values better compared to naltrindole. Although the study provides no stastistics to support this, it remains an interesting observation. Naltriben is known to possess a high selectivity for δ-2 receptors, and from this perspective it seems to confirm the results from our present study.

The question remains whether the use of a competitive δ-2 opioid antagonist, like naltriben (56), would have given different results than those obtained now with naltrindole 5′-isothiocyanate, a noncompetitive antagonist. The nature of an antagonist can be deduced from a plot with the negative logarithm of increasing doses of an agonist in the absciss and the relative response in a given system in the ordinate. Repeating the response curve with increasing doses of agonist in the presence of a competitive antagonist shifts the response curve to the right, while a noncompetitive antagonist progressively diminishes the maximal relative response. Only one agonist dose, the one resulting in a 100% response, was given in this study. Here, increasing doses of the δ-antagonists returned the initial response from sufentanil back to baseline. Whether sufentanil was irreversibily chased from the receptor (as with naltrindole 5′-isothiocyanate) or remained merely in competition with the antagonist (as with naltrindole) could not be determined with this experimental design. In our opinion, the same doses of antagonist could be tested, but then at least during different steady-state sufentanil serum levels. The results may nevertheless be hard to interpret, due to pharmacokinetic factors of the respective δ-antagonists.

Given the 100-fold higher binding affinity of sufentanil for μ-opiate sites compared to δ-opiate sites labeled by [^{3}H] [D-Ala^2,D-Leu^5]enkephalin (25), and the absence of high affinity specific binding of [^{3}H]-sufentanil to membranes of a neuroblastoma-glioma cell line known to bear high-affinity binding sites for enkephalin (28), the possibility that sufentanil binds strongly to δ-opiate receptors is extremely low. Because naltrindole has a relatively high affinity for μ-opiate receptors in vitro, being only 14 times less potent than naloxone (9,12), and given the large dose of naltrindole used in the present study, it may be possible that naltrindole had saturated μ-opiate receptors to a point that supraspinal antinociception could be blunted.

The differential antagonism by naltrindole 5′-isothiocyanate with respect to respiratory effects and antinociception evoked by intravenous sufentanil in rats suggests an involvement of δ-2 rather than δ-1 opiate receptors in reversing the respiratory depression induced by opioids. Whether a differential saturation of the low affinity type or the μ-2 opiate receptor did occur remains to be elucidated via crossover studies with antagonists highly selective for the respective opiate receptor subtypes. Evaluation of more than one subtype-mediated effect could further clarify the issue.

In conclusion, the data presented here indicate that respiratory depression following intravenous sufentanil in rats is selectively blocked by 10 mg/kg naltrindole 5′-isothiocyanate, a dose that leaves antinociception unaffected. The fact that a similar dose of naltrindole reverses both the sufentanil-induced antinociception and respiratory depression suggests that different sets of opiate receptor subtypes might be involved in the regulation of intravenous sufentanil-induced antinociception and respiratory depression.

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