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## Potentiation of the Analgesic Properties of Fentanyl-like Opioids with $\alpha_2$ -Adrenoceptor Agonists in Rats

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**Background:** Data on the analgesic properties of  $\alpha_2$  agonists and their interactions with opioids are conflicting. Experiments were conducted in rats to evaluate whether various available  $\alpha_2$  agonists can potentiate the analgesic properties of opioids and to determine the route of administration needed for optimal interaction.

**Methods:** The tail-withdrawal reaction test was used as an analgesia assay. In separate experiments, the interactions between systemic (subcutaneous, intravenous, and intraperitoneal) and spinally (epidural and intrathecal) administered  $\alpha_2$  agonists and opioids were evaluated. The antagonism of medetomidine plus fentanyl with naloxone and/or idazoxan also was studied.

**Results:** All  $\alpha_2$  agonists tested, when injected subcutaneously with fentanyl, potentiated the opioid-induced analgesia. In terms of a reduction of the amount of fentanyl needed to produce a deep surgical analgesia (tail-withdrawal reaction latency  $\geq 10$  s) over more than 2 h, the relative order of potency of the  $\alpha_2$  agonists tested was: medetomidine > dexmedetomidine > xylazine > clonidine > detomidine. For some of these  $\alpha_2$  agonists there was a biphasic effect; at the larger doses tested, a reduction in the fentanyl potentiation was present. The potentiation of the opioid activity with  $\alpha_2$  agonists was also demonstrated in terms of a longer duration of analgesia after combined treatment with fixed doses of opioids. The interaction between the  $\alpha_2$  agonists and the opioids remained present when a more profound criterion of analgesia (tail-withdrawal reaction latency  $\geq 30$  s) was used. Furthermore, the interactions between the  $\alpha_2$  agonists tested and fentanyl or sufentanil appeared to be independent of the route of administration. Potentiations were observed after simultaneous

subcutaneous injections of both groups of compounds, after the combination of intraperitoneal ( $\alpha_2$  agonist) plus subcutaneous (opioid), intravenous ( $\alpha_2$  agonist) plus epidural (opioid) and simultaneous epidural or intrathecal administrations. With regard to antagonism of the analgesic activity of combined treatment with  $\alpha_2$  agonists plus opioids, naloxone provided total antagonism, whereas the  $\alpha_2$  antagonist idazoxan overcame only the  $\alpha_2$  agonist-induced potentiation of fentanyl.

**Conclusions:** The tested  $\alpha_2$  agonists can potentiate the analgesic properties of opioids, but they have no intrinsic antinociceptive effects on spinal reflexes on their own. The potentiating activities of the  $\alpha_2$  agonists could be measured both in terms of a reduction of the amount of opioid needed to reach a particular level of analgesia and in terms of a longer duration of analgesia with a fixed dose of the opioid. The potentiations were observed with various  $\alpha_2$  agonists and opioids and appeared independent of the routes of administration. (Key words: Analgesics: opioids. Interactions (drug): opioids— $\alpha_2$  agonists. Sympathetic nervous system:  $\alpha_2$  agonists.)

INITIAL experiments in animals indicated that descending noradrenergic pathways play a role in the analgesic properties of opioids and that a major role in opioid- and stimulation-produced antinociception results from the activation of spinal  $\alpha_2$ -adrenergic receptors by noradrenergic neurons.<sup>1-6</sup> Since then, various studies have been conducted to study the role of  $\alpha_2$ -adrenoceptor agonists ( $\alpha_2$  agonists) in analgesia. Spinal administration of  $\alpha_2$  agonists, such as clonidine, medetomidine and ST-91, was shown to have antinociceptive properties or to potentiate the analgesic properties of opioids such as morphine in various animal species.<sup>1,4,7-12</sup> Also clinically, the interactions between  $\alpha_2$  agonists and opioids were mainly studied after epidural or intrathecal administration. Although on the basis of these clinical studies it is still debatable whether the  $\alpha_2$  agonists possess intrinsic analgesic properties, it is clear that spinally administered  $\alpha_2$  agonists can potentiate the analgesic properties of opioids and local anesthetics.<sup>1,3-17</sup>

Several experimental reports have pointed to a spinal site of action for the intrinsic analgesic- and opioid-

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potentiating effects of the  $\alpha_2$  agonists, leading to the hypothesis that  $\alpha_2$  agonists are more effective after spinal than after systemic administration.<sup>13,18-22</sup> However, in other clinical trials, a potentiation of the analgesic activity of opioids was also achieved with systemically administered  $\alpha_2$  agonists.<sup>18,23-27</sup> Therefore, it currently is not clear whether  $\alpha_2$  agonists should be given preferentially spinally instead of systemically to obtain the most effective potentiation of opioid analgesia.

To study this problem, we performed a series of experiments in rats to evaluate the interactions between  $\alpha_2$  agonists and opioids when given *via* various routes of administration. A comparison between various  $\alpha_2$  agonists also was included to compare these  $\alpha_2$  agonists with respect to their opioid-potentiating properties.

## Materials and Methods

### Animals

Approval from the Institutional Animal Care and Use Committee was obtained to perform the described experiments.

In all tests, male Wistar rats, weighing 200–230 g at the beginning of the tests were used. During testing, the animals were housed individually in standard observation cages equipped with a grid floor. For the recovery period after implantation of a spinal catheter (see below), the animals were housed in standard living cages with food and water *ad libitum*. All experiments and housing took place in a laboratory that was air conditioned (temperature  $21 \pm 1^\circ\text{C}$ ; relative humidity:  $65 \pm 5\%$ ) and continuously illuminated.

### Tail-withdrawal Reaction Procedure

The tail-withdrawal reaction (TWR) method used has been described in detail.<sup>28</sup> In brief, the rat was placed in a cylindrical rat holder with its tail hanging freely outside the cage. The distal 5 cm of the tail was immersed in a warm water bath ( $55 \pm 1^\circ\text{C}$ ) and the time for tail withdrawal was measured to the nearest 0.1 s. To minimize tissue damage at repeated testing, a cut-off time of 10 s was adopted.

### Other In Vivo Actions

The blockade of the pinna and cornea reflexes and muscular tone were scored as indices of supraspinal pharmacologic activity of the opioids.

Blockade of the pinna and cornea reflex are characteristic effects of the opioids at the level of the tenth

and fifth cranial nerves, whereas rigidity probably originates in the striatum and substantia nigra.<sup>29-31</sup> The pinna reflex consisted of a characteristic head twitch induced by a gentle mechanical stimulation of the inner ear with a blunt metal rod (diameter 0.5 mm). The response of the animal was scored from 0 (normal reflex) to 3 (absence of any motor response). Scores 1 and 2 indicate that the reflex was slightly or markedly obtunded, respectively. The cornea reflex was examined by stimulation of the eye by a gentle mechanical stimulation with a similar blunt metal rod and was also scored from 0 (normal reflex) to 3 (absence of any motor response). The scores given for skeletal muscle tone ranged from -3 (complete hypotonia) to 3 (lead pipe rigidity); a score of 0 documented normal tone and scores 1 and 2 weakly and moderately changed tone. In untreated control animals ( $n > 1,000$ ) a score  $\geq 2$  or  $\leq -2$  never occurred for any of these indexes.

### Spinal Catheterization

Epidural catheters were placed according to a technique described in detail elsewhere.<sup>32</sup> In brief, during general anesthesia, a polyethylene catheter (PE 10) was introduced into the epidural space over a length of 0.5 cm cephalad *via* a hole drilled in the fourth lumbar vertebra. Upon fixation of the catheter to the vertebra, the free end was tunneled subcutaneously toward the occiput. For intrathecal catheterization, an analogous procedure was used except that the catheter was brought into the intrathecal space through the dura. The animals were allowed 4 days to recover from anesthesia and surgery. During this time they had free access to food and water. Animals showing any sign of apparent neurologic damage were discarded. After the experiments, in which the animals were used only once, the rats were killed and the position of the catheter tip was verified at autopsy by an experienced investigator blind to the experimental results. Only the results from animals with catheter tips appropriately located in the epidural or intrathecal space, and without any sign of fibrinous tissue reaction around the catheter, were used for data analysis.

### Experiment 1

To assess the effects of a combined and concurrent treatment of medetomidine and fentanyl, rats were subcutaneously injected with saline plus increasing doses of fentanyl or with medetomidine plus fentanyl. Measurements were taken before and at 15-min intervals until 3 h after treatment. Dose-response functions

$\alpha_2$ -AGONIST-OPIOID POTENTIATIONS

were determined for fentanyl in the presence of 0.00, 0.04, 0.16, 0.63, and 2.50 mg/kg medetomidine. Based on these dose-response curves, median effective doses ( $ED_{50}$ s) for a TWR latency  $\geq 10.0$  s at 60 and 120 min after treatment were assessed.

*Experiment 2*

To document the potentiating effects of various  $\alpha_2$  agonists of the opioid analgesia,  $ED_{50}$ s of fentanyl were determined for a TWR latency  $\geq 10.0$  s at 2 h after subcutaneous administration in the presence of xylazine (0.63, 2.50, 10, or 40 mg/kg), clonidine (0.04, 0.16, 0.63, or 2.50 mg/kg), detomidine (0.04, 0.16, 0.63, or 2.50 mg/kg), or dexmedetomidine (0.16, 0.63, or 2.50 mg/kg). The data obtained after combination of fentanyl with medetomidine (0.04, 0.16, 0.63, or 2.50 mg/kg) and saline are those of experiment 1.

To control for the intrinsic analgesic effects of the  $\alpha_2$  agonists, separate experiments were conducted using the various test doses mentioned above. With none of the test conditions was a TWR latency  $> 6.0$  s observed. However, on some occasions, a doubling of the baseline TWR latency was present. Because at no time was the selection criterion of a TWR latency  $> 6.0$  or  $\geq 10.0$  s was reached, two criterion values always used to validate this test procedure,<sup>28</sup> the data on the intrinsic effects of the  $\alpha_2$  agonists are not further reported here.

*Experiment 3*

To evaluate whether the interactions between  $\alpha_2$  agonists and opioids remained present when a more stringent criterion of analgesia and different routes of administration were used, interactions were measured between medetomidine and fentanyl using a TWR latency  $\geq 30$  s as a criterion, in the following way. Dose-response after fentanyl was determined in the presence of medetomidine (0.00, 0.04, 0.16, or 0.63 mg/kg). Medetomidine was given intraperitoneally 15 min before the subcutaneous treatment with fentanyl. Measurements were taken before the start of the injections and 30, 60, 90, and 120 min after the last injection of fentanyl.

*Experiment 4*

To assess the interactions between intravenous injected  $\alpha_2$  agonists and spinally administered opioids, medetomidine (0.00, 0.031, 0.063, or 1.25 mg/kg) was injected intravenously 15 min before epidural administration of varying doses of sufentanil. Measure-

ments were performed 5, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 175, and 180 min after the epidural administration.

*Experiment 5*

To evaluate the interactions between opioids and  $\alpha_2$  agonists after concurrent spinal administration, the effects of sufentanil, clonidine and sufentanil plus clonidine were evaluated after epidural and intrathecal administration. Dose-response functions were generated for sufentanil in the presence of the vehicle and 80  $\mu$ /rat clonidine, and for clonidine mixed with vehicle and 0.063  $\mu$ /rat sufentanil. Measurements were taken 5, 10, 15, 30, 45, and 60 min after the spinal administration of the compounds in a fixed diluent volume of 10  $\mu$ l. Epidural and intrathecal injections of clonidine alone at doses as great as 640  $\mu$ g/rat did not result in a TWR latency  $> 6.0$  s. Comparable to the systemic treatments with  $\alpha_2$  agonists, however, a doubling of the baseline TWR latencies was present in some of the animals. Because the criterion of activity was not reached, the results of spinal clonidine alone are not reported in their entirety here.

*Experiment 6*

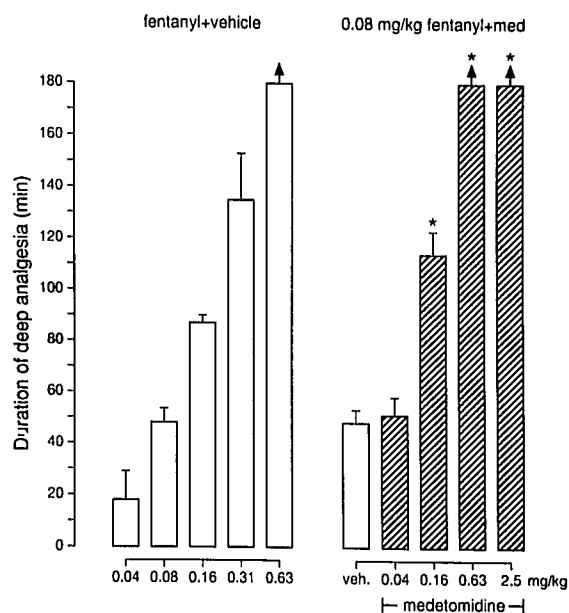
To confirm the opioid and  $\alpha_2$  agonists characteristics of the interaction of a combined treatment of medetomidine and fentanyl, both drugs were injected subcutaneously at a dose of 0.16 mg/kg each. Thirty-five minutes later, the animals were treated with various subcutaneous doses of naloxone, idazoxan, or naloxone plus idazoxan. Behavior testing was performed 5, 15, 30, . . . 135, 150, and 180 min after the last treatment.

*Drugs*

Clonidine HCl, detomidine HCl, dexmedetomidine HCl, fentanyl citrate, idazoxan HCl, medetomidine HCl, naloxone HCl, sufentanil citrate and xylazine HCl were freshly prepared as aqueous solutions. For subcutaneous and intraperitoneal injections, the drugs were given in a volume of 1 ml/100 g body weight. Intravenously, 0.2 ml/100 g body weight was used, and spinally a fixed diluent volume of 10  $\mu$ l was always used. Test doses were selected from the geometric series 0.01, 0.04, . . . 10, 40 mg/kg or from the series 0.031, 0.063, . . . 20, 40 mg/kg. In all experiments, five animals were used at each treatment condition.

*Data Analysis*

Criterion values were defined for each of the four variables that were examined. The TWR latency was



**Fig. 1.** Duration of analgesia obtained with various doses of fentanyl in combination with saline vehicle (*left*) and 0.08 mg/kg fentanyl with various doses of medetomidine (*right*). Each result represents the mean ( $\pm$ SEM) duration (minutes) of a tail-withdrawal reaction latency  $\geq 10$  s in five rats. Measurements were performed until 3 h after subcutaneous treatment. Differences between saline and medetomidine treated groups were evaluated with the two-tailed Mann-Whitney *U* test: \**P* < 0.05.

evaluated with a TWR latency > 6.0 s (mild analgesia) or a TWR latency  $\geq 2$  or  $\leq -2$  were taken into account for data analysis. For each treatment condition, results were expressed as the number of animals meeting cri-

terion. On these data ED<sub>50</sub> values and 95% confidence limits were calculated according to Finney's iterative method.<sup>33</sup> Differences in duration of analgesia between experimental conditions were evaluated by the Mann-Whitney *U* test (two-tailed<sup>34</sup>).

## Results

### Experiment 1

The mean ( $\pm$ SEM) duration of analgesia obtained with increasing doses of subcutaneous administered fentanyl is given in figure 1 (left). Fentanyl produced a dose-related increase in the duration of analgesia from 18.0 ( $\pm 11.02$ ) min with 0.04 mg/kg to >180 min after 0.63 mg/kg fentanyl. Based on these results, the ED<sub>50</sub>s of fentanyl for a TWR latency  $\geq 10.0$  s at 1 and 2 h after treatment were 0.074 and 0.30 mg/kg, respectively (table 1). Addition of medetomidine to fentanyl increased the duration of analgesia obtained with fixed doses of fentanyl (fig. 1; table 2). As illustrated on the right hand panel of figure 1, the duration of analgesia obtained with 0.08 mg/kg fentanyl increased from 48.0 ( $\pm 5.61$ ) to 114.0 ( $\pm 9.0$ ) and >180 min by adding 0.16 and 0.63 or 2.5 mg/kg medetomidine. Already with 0.04 mg/kg medetomidine, a prolongation of the duration of analgesia of 0.31 mg/kg fentanyl was present (table 2). From doses  $\geq 0.16$  mg/kg medetomidine onward, a TWR latency  $\geq 10.0$  s was obtained with 0.02 mg/kg fentanyl, a dose that when given alone was inactive. The maximal potentiation of fentanyl in terms of an increase in duration of analgesia was measured

**Table 1.** ED<sub>50</sub> (plus 95% confidence limits) of Fentanyl for a Tail Withdrawal Latency (TWR)  $\geq 10.0$  s at 60 and 120 min after Treatment, for a Blockade of the Pinna and Cornea Reflexes and for the Induction of Muscle Rigidity

Treatment	TWR $\geq 10$ s at 60 min	TWR $\geq 10$ s at 120 min	Blockade Pinna Reflex	Blockade Cornea Reflex	Induction of Muscle Rigidity
Vehicle	0.074 (0.049–0.11)	0.30 (0.22–0.40)	0.085 (0.063–0.12)	0.13 (0.095–0.17)	0.11 (0.091–0.14)
Medetomidine					
0.04 mg/kg	0.085 (0.063–0.12)	0.17 (0.13–0.23)	0.028 (0.021–0.038)	0.11 (0.065–0.19)	>2.50
0.16 mg/kg	0.019 (0.014–0.025)	0.064 (0.043–0.096)	0.016 (0.011–0.024)	0.024 (0.018–0.033)	>2.50
0.63 mg/kg	0.007 (0.0047–0.011)	0.014 *(0.0094–0.021)	0.0061 (0.0045–0.0083)	0.007 (0.0057–0.0087)	>2.50
2.50 mg/kg	0.028 (0.019–0.042)	0.049 (0.036–0.066)	0.0081 (0.0054–0.012)	0.0093 (0.0062–0.014)	>2.50

Fentanyl was injected subcutaneously at T0 in combination with different doses of medetomidine. All doses are given in mg/kg and based on the results of five different rats per test condition.

$\alpha_2$ -AGONIST-OPIOID POTENTIATIONS**Table 2. Duration of Analgesia Obtained with Different Doses of Fentanyl in Combination with Vehicle or Various Doses of Medetomidine**

Pretreatment	Dose of Fentanyl (mg/kg)					
	0.02	0.04	0.08	0.16	0.31	0.63
Vehicle	0.0 ± 0.0	18.0 ± 11.0	48.0 ± 5.6	87.0 ± 3.0	135.0 ± 19.0	>180.0
Medetomidine						
0.04 mg/kg	—	—	51.0 ± 7.7	93.0 ± 17.4	>180.0*	—
0.16 mg/kg	54.0 ± 23.5*	105.0 ± 17.4*	114.0 ± 9.0*	171.0 ± 3.7*	>180.0*	—
0.63 mg/kg	111.0 ± 12.2*	135.0 ± 6.7*	>180.0*	>180.0*	—	—
2.50 mg/kg	18.0 ± 12.0*	87.0 ± 29.4*	>180.0*	—	—	—

Values represent the average  $\pm$ SEM duration (minutes) of a TWR  $\geq 10.0$  s of five rats. Doses are expressed in mg/kg. All injections (pretreatment plus fentanyl) were combined injected subcutaneously at the start of the experiment. Measurements were performed up to 3 h after subcutaneous treatment. Differences between saline and medetomidine treated groups were evaluated with the Mann-Whitney U test (two-tailed): \* $P < 0.05$ .

with 0.63 mg/kg medetomidine. With 2.50 mg/kg medetomidine, the potentiation of the average durations of analgesia started to decline again, especially at the lowest doses of fentanyl. Based on the increases in duration of a TWR latency  $\geq 10.0$  s after combinations of medetomidine plus fentanyl, there was a reduction in the ED<sub>50</sub>s of fentanyl needed to obtain analgesia after 60 and 120 min (table 1). Increasing the doses of medetomidine from 0.04 to 0.63 mg/kg resulted in a decrease of the ED<sub>50</sub>s of fentanyl from 0.085 to 0.007 and from 0.17 to 0.014 mg/kg, at 60 and 120 min, respectively. As observed for the durations of analgesia, with 2.50 mg/kg medetomidine somewhat higher ED<sub>50</sub>s of fentanyl were needed. Hence also here, a slight reversal in the potentiating properties of medetomidine at the highest dose tested was seen.

Besides analgesia, the ED<sub>50</sub>s for the blockade of the pinna and cornea reflexes and the induction opioid-induced muscle rigidity were assessed. For fentanyl, these ED<sub>50</sub>s were 0.085, 0.13, and 0.11 mg/kg, respectively. With regard to the blockade of the pinna and cornea reflexes, adding medetomidine to fentanyl resulted in a reduction of the ED<sub>50</sub>s similar to those observed for analgesia (table 1). With regard to muscle tone, the addition of medetomidine overcame the fentanyl-induced rigidity; often even a hypotonia was measured.

**Experiment 2**

To compare the opioid potentiating properties of the  $\alpha_2$  agonists tested, ED<sub>50</sub>s of fentanyl for a TWR latency  $\geq 10$  s at 120 min after treatment, were assessed in combination with various doses of the  $\alpha_2$  agonists (table 3). With increasing doses of xylazine, there was a dose-related reduction in the ED<sub>50</sub>s of fentanyl. The ED<sub>50</sub> of

fentanyl decreased from 0.22 to 0.056 mg/kg as the dose of xylazine increased from 0.63 to 40.00 mg/kg. With 40 mg/kg xylazine, the ED<sub>50</sub> of fentanyl was reduced by a factor of 5.4. With clonidine, the strongest potentiation of fentanyl was observed with 0.63 mg/kg clonidine. At this dose, the ED<sub>50</sub> of fentanyl decreased to 0.074 mg/kg; a 4.05-fold reduction. At

**Table 3. ED<sub>50</sub> of Fentanyl for a Tail Withdrawal Latency (TWR)  $\geq 10.0$  s at 120 min after Treatment with Different Doses of  $\alpha_2$  Agonists**

Treatment Compound	Dose (mg/kg)	ED <sub>50</sub> Fentanyl for TWR $\geq 10$ s	Ratio
Saline	—	0.30 (0.22–0.40)	1.00
Xylazine	0.63	0.22 (0.15–0.34)	1.36
	2.50	0.17 (0.11–0.27)	1.76
	10.0	0.11 (0.091–0.14)	2.73
	40.0	0.056 (0.045–0.069)	5.36
Clonidine	0.04	0.17 (0.11–0.25)	1.76
	0.16	0.097 (0.065–0.15)	3.09
	0.63	0.074 (0.055–0.10)	4.05
	2.50	$\geq 0.16$	<1.88
Detomidine	0.04	0.13 (0.095–0.17)	2.31
	0.16	0.22 (0.15–0.34)	1.36
	0.63	0.13 (0.08–0.21)	2.31
	2.50	0.64 (0.048–0.87)	0.47
Medetomidine	0.04	0.17 (0.13–0.23)	1.76
	0.16	0.064 (0.043–0.096)	4.69
	0.63	0.014 (0.0094–0.021)	21.43
	2.50	0.049 (0.036–0.066)	6.12
Dexmedetomidine	0.16	0.097 (0.071–0.13)	3.09
	0.63	0.043 (0.028–0.064)	6.98
	2.50	$\geq 0.04$ (toxic!)	—

The combined treatments were injected subcutaneously at T0. The ratio represents the division of the ED<sub>50</sub> of fentanyl plus saline to that of fentanyl plus another drug condition. Doses are given in mg/kg and are based on the results obtained with five rats at each different treatment condition.

higher doses of clonidine, less potentiation of fentanyl was observed, with higher  $ED_{50}$ s as a consequence. Also with detomidine and medetomidine biphasic effects were found. With 0.63 mg/kg detomidine and medetomidine, the  $ED_{50}$ s of fentanyl decreased to 0.13 and 0.014 mg/kg, respectively. There was thus a 2.31- and 21.43-fold reduction in the  $ED_{50}$  of fentanyl. With 2.5 mg/kg detomidine and medetomidine, the  $ED_{50}$ s of fentanyl increased again to 0.64 and 0.049 mg/kg. Hence, increasing the doses of both detomidine and medetomidine above 0.63 mg/kg resulted in a reduced potentiation of the analgesic properties of fentanyl. Also with dexmedetomidine the lowest  $ED_{50}$  of fentanyl was measured with 0.63 mg/kg dexmedetomidine, being 0.043 mg/kg. With 2.5 mg/kg dexmedetomidine, no  $ED_{50}$  value of fentanyl could be determined because the majority of animals died after combined treatments with doses  $\geq 0.04$  mg/kg fentanyl.

### Experiment 3

To evaluate whether the interaction between  $\alpha_2$  agonists and opioids remained present when a more stringent criterion of analgesia and different routes of administration were used, interactions were measured between intraperitoneally administered medetomidine and subcutaneous fentanyl with the criterion of a TWR latency  $\geq 30$  s for analgesia. In saline-pretreated rats, the  $ED_{50}$ s of fentanyl to reach a TWR latency  $\geq 30$  s at 30, 60, 90, and 120 min after treatment were 0.085 (0.053–0.14), 0.11 (0.091–0.14), 0.13 (0.095–0.17) and 0.34 (0.25–0.46) mg/kg, respectively (fig. 2). A four-fold increase in fentanyl was needed to prolong the duration of a TWR latency  $\geq 30$  s from 30 to 120 min. With medetomidine at doses as great as 0.63 mg/kg, a dose-related reduction in the  $ED_{50}$ s of fentanyl was observed. With 0.63 mg/kg medetomidine, the corresponding  $ED_{50}$ s of fentanyl at the various periods were 0.012 (0.090–0.017), 0.012 (0.082–0.018), 0.024 (0.018–0.033) and 0.024 (0.018–0.033) mg/kg. When the  $ED_{50}$ s of fentanyl plus saline are compared with those of fentanyl plus 0.63 mg/kg medetomidine, 7.08-, 9.17-, 5.40- and 14.17 -fold differences in the  $ED_{50}$ s of fentanyl were observed at the various time points. The strongest potentiation of the fentanyl with 0.63 mg/kg medetomidine, and thus the largest differentiation between the  $ED_{50}$ s of fentanyl plus saline *versus* fentanyl plus 0.63 mg/kg medetomidine, was observed 120 min after treatment. These results thus indicate that the potentiation can be optimally evaluated

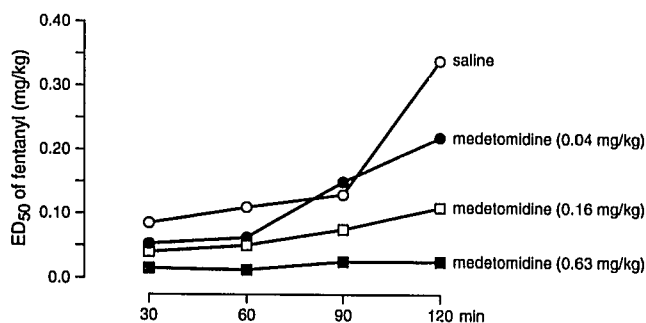


Fig. 2. Median effective doses ( $ED_{50}$ s) of fentanyl for a tail-withdrawal reaction latency  $\geq 30$  s at various periods after combined treatment with saline or various doses of medetomidine. Saline or medetomidine was injected 15 min before treatment with subcutaneous fentanyl.

at time points where analgesia starts to wear off under control conditions.

### Experiment 4

To test whether intravenously injected  $\alpha_2$  agonists can potentiate the activity of spinally administered opioids, interactions between intravenously administered medetomidine and epidurally injected sufentanil were studied. The  $ED_{50}$ s of sufentanil for a TWR latency  $\geq 10$  s at 60 and 90 min after epidural administration were 2.34 and 4.08  $\mu$ g/rat. Intravenous co-administration of medetomidine reduced the  $ED_{50}$ s of sufentanil in a dose-related manner at the doses tested here (table 4). With 0.125 mg/kg medetomidine, the  $ED_{50}$ s of sufentanil decreased to 0.67 and 1.17  $\mu$ g/rat at 60 and 90 min, respectively. A potentiation of sufentanil's activity was also detected in terms of the blockade of the pinna and cornea reflexes. The  $ED_{50}$ s of sufentanil for the blockade of the pinna and cornea reflexes decreased from 0.51 and 1.34  $\mu$ g/rat for sufentanil (plus vehicle) to 0.26 and 0.26  $\mu$ g/rat when sufentanil was combined with 0.125 mg/kg intravenous medetomidine (table 4). Similar to the interactions of fentanyl and medetomidine after systemic administration in experiment 1, the addition of intravenous medetomidine to epidural sufentanil completely reversed the opioid-induced muscle rigidity. Even a tendency to a muscle hypotonus was present.

The potentiation of the analgesic effects of epidural sufentanil with intravenously administered medetomidine is also reflected in the increased time that a TWR latency  $\geq 10.0$  s could be observed after a single dose of sufentanil. Epidural sufentanil plus vehicle produces a dose-related increase in the duration of analgesia from

$\alpha_2$ -AGONIST-OPIOID POTENTIATIONS**Table 4. ED<sub>50</sub> of Sufentanil (in  $\mu\text{g}/\text{rat}$ ) for a Tail Withdrawal Latency (TWR)  $\geq 10.0$  s at 60 and 90 min after Treatment, for a Blockade of the Pinna and Cornea Reflexes and for the Induction of Muscle Rigidity**

Pretreatment	TWR $\geq 10$ s at 60 min	TWR $\geq 10$ s at 90 min	Blockade Pinna Reflex	Blockade Cornea Reflex	Induction of Muscle Rigidity
Vehicle	2.34 (1.56–3.50)	4.08 (3.01–5.52)	0.51 (0.34–0.76)	1.34 (0.99–1.82)	1.55 (1.03–2.31)
Medetomidine 0.031 mg/kg	2.34 (1.56–3.50)	4.68 (3.01–5.52)	0.51 (0.38–0.69)	1.35 (0.73–1.90)	>10.0
0.063 mg/kg	1.77 (1.44–2.18)	4.68 (3.13–7.00)	0.59 (0.43–0.79)	0.67 (0.50–0.91)	>5.00
0.125 mg/kg	0.67 (0.45–1.00)	1.17 (0.73–1.89)	0.26 (0.19–0.35)	0.26 (0.19–0.35)	>5.00

Sufentanil was injected epidurally at 15 min after the intravenous pretreatment with either vehicle or medetomidine (in mg/kg). Data are based on the results of five rats per treatment condition.

13 ( $\pm 5.15$ ) to 93.0 ( $\pm 5.61$ ) min as the dose increases from 0.63 to 5.00  $\mu\text{g}/\text{rat}$  (fig. 3, left, open columns). The addition of intravenously administered medetomidine increased the duration of analgesia. With 0.125 mg/kg medetomidine, for instance, the durations of analgesia with 0.63, 1.25, 2.50, and 5.00  $\mu\text{g}/\text{rat}$  sufentanil were 66.0 ( $\pm 13.1$ ), 81.0 ( $\pm 14.7$ ), 105.0 ( $\pm 10.6$ ), and 135.0 ( $\pm 19.6$ ) min, respectively (fig. 3).

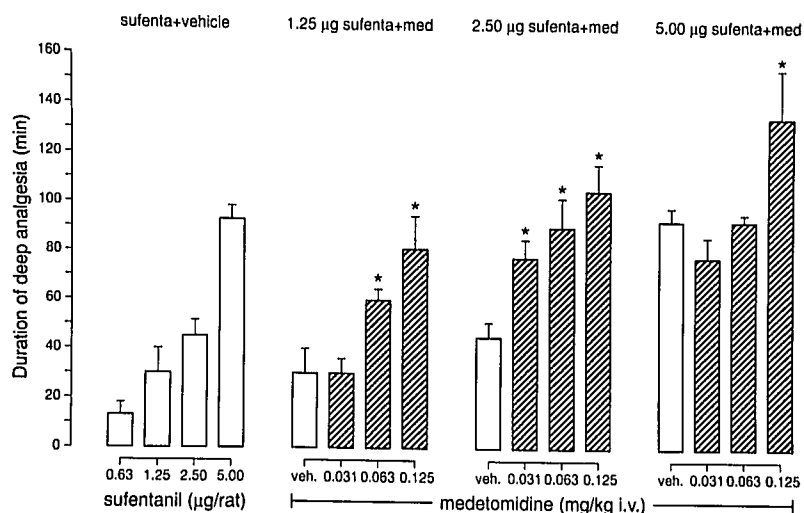
**Experiment 5**

To evaluate the interaction between  $\alpha_2$  agonists and opioids at a spinal level, we tested the interactions between clonidine and sufentanil after both epidural and intrathecal administration. Sufentanil produced a dose-related analgesic activity after both epidural and in-

trathecal administration with lowest ED<sub>50</sub>s of 0.22  $\mu\text{g}/\text{rat}$  (table 5). The ED<sub>50</sub>s of sufentanil for a blockade of the pinna and cornea reflexes and for muscle rigidity were 0.39, 0.34, and 0.44  $\mu\text{g}/\text{rat}$  epidurally and 0.34, 0.29 and 0.34  $\mu\text{g}/\text{rat}$  intrathecally. With both epidural and intrathecal clonidine at concentrations as great as 640  $\mu\text{g}/\text{rat}$ , no analgesia was found. The only effect observed with spinal clonidine was a strong reduction in muscle tone with lowest ED<sub>50</sub>s of 226 and 171  $\mu\text{g}/\text{rat}$  after epidural and intrathecal administration, respectively.

Combined with 80  $\mu\text{g}$  clonidine, the lowest ED<sub>50</sub>s of sufentanil for TWR latency  $\geq 10$  s and a blockade of the pinna and cornea reflexes decreased to respectively 0.067, 0.12, and 0.058  $\mu\text{g}/\text{rat}$  after epidural administration and 0.067, 0.12, and 0.058  $\mu\text{g}/\text{rat}$  after in-

**Fig. 3. Average duration of analgesia after intravenous medetomidine (med) and epidural sufentanil. Each result represents the average ( $\pm$ SEM) duration (minutes) of a tail-withdrawal reaction latency  $\geq 10.0$  s in five rats. Medetomidine or saline vehicle (open columns) was injected intravenously 15 min before the epidural administration of sufentanil. Differences between saline- and medetomidine-treated groups were evaluated with the Mann-Whitney *U* test: \**P* < 0.05.**



**Table 5. Characteristics of Analgesia and Other *In vivo* Pharmacologic Effects of Epidurally and Intrathecally Administered Clonidine and Sufentanil in the Rat**

Parameter	CLO	SUF	SUF/CLO 80	CLO/SUF 0.063
Epidural administration				
TWR $\geq$ 10.0 s	>640	0.22 (0.15–0.34)	0.067 (0.049–0.091)	149.30 (86.70–257.1)
Blockade pinna reflex	>640	0.39 (0.29–0.53)	0.12 (0.090–0.14)	>640
Blockade cornea reflex	>640	0.34 (0.22–0.50)	0.058 (0.043–0.080)	74.60 (43.30–128.5)
Muscle tonus rigidity	—	0.44 (0.30–0.66)	—	—
Muscle tonus hypotonia	226.4 (187.7–279.0)	—	—	—
Intrathecal administration				
TWR $\geq$ 10.0 s	>640	0.22 (0.18–0.27)	0.067 (0.044–0.10)	149.3 (82.00–271.8)
Blockade pinna reflex	>640	0.34 (0.22–0.50)	0.12 (0.086–0.16)	>640
Blockade cornea reflex	>640	0.29 (0.20–0.44)	0.058 (0.043–0.079)	85.70 (47.10–156.1)
Muscle tonus rigidity	—	0.34 (0.22–0.50)	—	—
Muscle tonus hypotonia	171.6 (126.7–232.5)	—	—	—

The table represents the lowest ED<sub>50</sub> (and 95% confidence limits) in  $\mu\text{g}/\text{rat}$  of the different treatments for a tail withdrawal latency (TWR)  $\geq$  10.0 s, for the blockade of the pinna and cornea reflexes, and for the induction of either muscle rigidity or hypotonia of clonidine (CLO), sufentanil (SUF), of sufentanil in the presence of a fixed dose of 80  $\mu\text{g}/\text{rat}$  clonidine (SUF/CLO 80) and of clonidine in the presence of a fixed dose of 0.063  $\mu\text{g}/\text{rat}$  sufentanil (CLO/SUF 0.063). At all doses used to calculate the ED<sub>50</sub> data on five rats were collected. — = the treatment does not reach the criterion of activity.

trathecal administration. When injected together with a fixed dose of 0.063  $\mu\text{g}/\text{rat}$  sufentanil, the corresponding ED<sub>50</sub>s of clonidine after epidural and intrathecal administration were, respectively, 149.3 > 640, 74.6, and 149.3 > 640 and 85.7  $\mu\text{g}/\text{rat}$ . With regard to muscle tone, the combination of sufentanil plus clonidine always resulted in a weak muscle hypotonia (scores varying between -1 and -2).

#### Experiment 6

The antagonism of the combined treatment of medetomidine plus fentanyl with naloxone, idazoxan and naloxone plus idazoxan in terms of duration of analgesia is presented in figure 4. Naloxone reduced the average duration of analgesia of 0.16 mg/kg fentanyl plus 0.16 mg/kg medetomidine in a dose-related manner. The duration of analgesia of fentanyl plus medetomidine diminished from 114.0 ( $\pm$ 6.0) to 0.0 ( $\pm$ 0.0) min as the dose of naloxone increased from 0.0025 to 0.63 mg/kg. Idazoxan reduced ( $P < 0.05$ ) the duration of analgesia at doses varying between 0.04 and 10 mg/kg. At no time was a complete antagonism of the analgesic activity observed and no differences with the saline plus fentanyl-treated group was apparent ( $P > 0.05$ ). Idazoxan thus only overcame the medetomidine-induced potentiation of fentanyl. A combined treatment of 0.01 mg/kg naloxone plus 0.04 mg/kg idazoxan reduced ( $P < 0.05$ ) the duration of the fentanyl plus medetomidine-induced analgesia to a duration observed with fentanyl alone. Combinations of higher

doses of both antagonists were more effective, and with 0.16 mg/kg naloxone plus 0.63 mg/kg idazoxan all behavioral effects induced by 0.16 mg/kg fentanyl plus medetomidine were blocked.

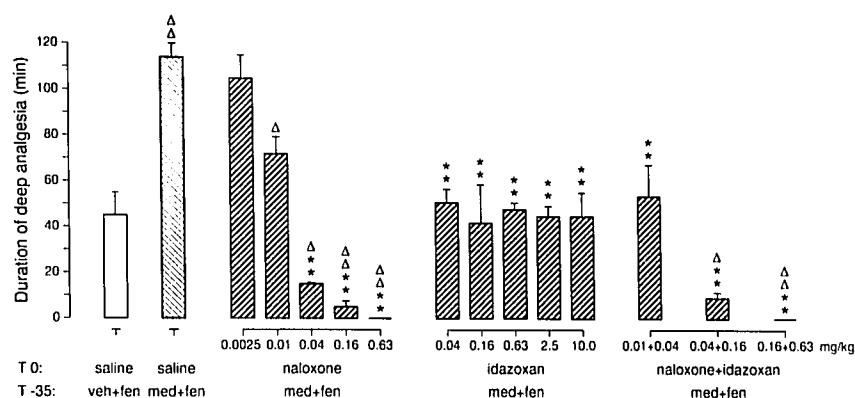
#### Discussion

In the various experiments described here, a potentiation of the analgesic properties of opioids through  $\alpha_2$ -adrenergic agonists ( $\alpha_2$  agonists) was observed. These results thus clearly confirm the interactive role between these two classes of drugs (see Introduction). However, as opposed to some earlier reports in animals,<sup>7,35–37</sup> no analgesic properties were measured with the  $\alpha_2$  agonists alone, not even after spinal administration. Differences in the experimental procedures, the use of a TWR test in our experiments *versus*, for instance, a hot-plate test, may account for the differences in outcome observed. Using systemic  $\alpha_2$  agonists such as medetomidine, Pertovaara *et al.*<sup>37</sup> also failed to demonstrate a positive analgesic activity in the TWR test. In a variety of other tests, such as the formalin test, the hot-plate test, and the righting test, all analgesia assays based on complex motor behavioral responses,<sup>38</sup>  $\alpha_2$  agonists have been demonstrated to diminish motor responding without affecting the reflex activity, if additionally measured. As reviewed by Vonvoightlander,<sup>38</sup> the pharmacologic selectivity of these more complex motor behavioral tests is much lower than that of the thermal spinal reflex models. As a consequence of the



$\alpha_2$ -AGONIST-OPIOID POTENTIATIONS

Fig. 4. Antagonism of the analgesic properties of 0.16 mg/kg medetomidine (med) plus 0.16 mg/kg fentanyl (fen) with naloxone and idazoxan. Each result represents the average ( $\pm$ SEM) duration (minutes) of a tail-withdrawal reaction latency  $\geq 10$  s in five rats. The drugs were injected subcutaneously 35 min before (medetomidine and fentanyl) or at the beginning (naloxone and idazoxan) of the experiment. Differences with regard to the saline plus fentanyl condition ( $\Delta$ ) and the medetomidine plus fentanyl condition (\*) were evaluated with the Mann-Whitney *U* test: \**P* < 0.05;  $\Delta$ *P* < 0.05; \*\**P* < 0.01;  $\Delta\Delta$ *P* < 0.01.



reduced selectivity, more false-positive effects are observed with different pharmacologic agents, including benzodiazepines, barbiturates, neuroleptics and  $\alpha$ -adrenergic agents. In the TWR tests, in which activity was demonstrated with  $\alpha_2$  agonists, either a much weaker criterion for the definition of analgesia was used (e.g., a doubling of baseline latencies,<sup>39-41</sup> or a percentage of maximal possible effect between 50 and 80%<sup>42</sup>) or animals were anesthetized with halogenated vapors,<sup>43</sup> which can interfere with the possible analgesic and anesthetic effects of the tested agents. Because the TWR test is originally evaluated using criterion values of a TWR latency > 6.0 and  $\geq 10.0$  s, and because with these criterion rank-correlations with clinical efficacy and opiate receptor occupancy have been established,<sup>28,44-50</sup> one can not simply adjust the criterion for analgesia within this test procedure without doing a completely new test evaluation.

No pre- or postoperative analgesia has been reported up to now with the clinical use of  $\alpha_2$  agonists as sole analgesic agents. In the clinical studies in which spinal administration of clonidine was reported to produce pain-relieving effects, the drug was given shortly after general anesthesia or after a loading or test dose with a local anesthetic, or analgesics were used as rescue medication.<sup>17,51-57</sup> Because of possible interactions between clonidine and these analgesics and anesthetics, it is not possible to attribute the observed pain-relieving effects simply to clonidine alone. Even in the study of Aho *et al.*,<sup>58</sup> using dexmedetomidine for postoperative pain relief after laparoscopic tubal ligation, no distinction can be made between analgesia and sedation.

The interaction between  $\alpha_2$  agonists and opioids was seen with various  $\alpha_2$  agonists combined with two opioids (fentanyl and sufentanil). The  $\alpha_2$  agonists revealed some differences with regard to their opioid-

potentiating properties (see table 2). In terms of potency, defined as a reduction in the amount of fentanyl needed to produce a deep surgical analgesia for more than 2 h, the rank order of the  $\alpha_2$  agonists tested at their optimal dose was: medetomidine > dexmedetomidine > xylazine > clonidine > detomidine. This order of potency does not simply correlate with the selectivity of these  $\alpha_2$  agonists for central  $\alpha_2$ -adrenoceptor sites, as reported by Maze and Tranquilli.<sup>59</sup> Interactions at different  $\alpha_2$ -adrenergic subtype sites but also interactions at  $\alpha_1$ -adrenergic sites<sup>60</sup> and other binding sites, such as the imidazoline sites,<sup>60,61</sup> may contribute to the differences in activity between these  $\alpha_2$  agonists. The nonselectivity of some of these agents was also reflected in the reductions of the opioid potentiating properties at larger doses. With clonidine, detomidine and medetomidine, a reduction in the fentanyl-potentiating activity was observed with 2.50 mg/kg, the largest dose tested. With xylazine, such a biphasic activity was not present at doses of as much as 40 mg/kg. Larger doses were not tested, because with 40 mg/kg there already was very pronounced hypotonia. Also with dexmedetomidine, biphasic activity was absent because of the toxicity with combinations of doses  $\geq 2.50$  mg/kg dexmedetomidine plus fentanyl. Because no lethality was observed during testing with dexmedetomidine alone, the toxicity observed in this study appears to be due to the combination of small doses of fentanyl with dexmedetomidine. Because of the toxicity observed with dexmedetomidine plus fentanyl, the relative potency of this agent could have been misjudged. Therefore, the rank order presented above includes the *in vivo* safety aspect of the interaction opioid/ $\alpha_2$  agonist in rats.

The potentiation of the opioid activity with  $\alpha_2$  agonists was also seen on the opioid-induced blockade of

the pinna and cornea reflexes, two supraspinal side effects of the opioids. With regard to the muscle tone, the combination of  $\alpha_2$  agonists plus opioids resulted mainly in a weak hypotonia instead of the normal opioid-induced rigidity.

The potentiation of the analgesic activity of the opioids with the  $\alpha_2$  agonists tested was not due simply to secondary behavioral effects of the  $\alpha_2$  agonists. The  $\alpha_2$  agonist-induced muscle hypotonia and hypothermia were by themselves not sufficient conditions to result in a false positive activity when tested alone in the TWR test. Furthermore, when the criterion for analgesia was increased from a TWR latency  $\geq 10$  s to  $\geq 30$  s, as demonstrated in experiment 3, the potentiating activity of the  $\alpha_2$  agonists remained present. Sedation by itself, as for instance also obtained with 40 mg/kg of the benzodiazepine chlordiazepoxide or the barbiturate pentobarbital, does not result in a TWR latency  $> 6.0$  s (unpublished data). With regard to hypothermia, Lichtman *et al.*<sup>62</sup> recently, again, demonstrated that the tail flick latency appears independent of changes in tail-skin temperature or core temperature.

Important for interactions to be used in analgesia is the existence of an antagonist. As demonstrated in experiment 6 (fig. 4), naloxone is able to completely block the analgesia induced by the opioid fentanyl plus the  $\alpha_2$  agonist medetomidine. The  $\alpha_2$  antagonist idazoxan only blocked the medetomidine-induced potentiation of the opioid analgesic activity. Because the opioid antagonist naloxone, but not the  $\alpha_2$  antagonist idazoxan, was able to completely antagonize the analgesic properties of medetomidine plus fentanyl, it appears that the analgesia, as measured here with the TWR test, is primarily opioid-mediated. The role of the  $\alpha_2$  agonists consists of potentiating the opioid analgesic activity.

To evaluate whether there would be any preferential route of administration for the  $\alpha_2$  agonists to potentiate opioid analgesia, various systemic and spinal treatment modalities were tested. A potentiation of the opioid analgesic activity with  $\alpha_2$  agonists was observed when (1) the  $\alpha_2$  agonists and the opioids were given simultaneously in a subcutaneous injection (experiments 1 and 2); (2) the  $\alpha_2$  agonist was injected intraperitoneally 15 min before a subcutaneous injection of the opioid (experiment 3); (3) the  $\alpha_2$  agonist was injected intravenously 15 min before an epidural administration of the opioid (experiment 4) and (4) the  $\alpha_2$  agonists and the opioids were simultaneously injected in the intrathecal or the epidural space (experiment 5). There-

fore, independent of the route of administration, a clear potentiation of analgesic activity of the opioids with the tested  $\alpha_2$  agonists was present. These results thus illustrate that the  $\alpha_2$  agonists do not necessarily have to be administered spinally to potentiate opioid analgesics (see introduction). These results therefore confirm a series of recent clinical studies demonstrating no major differences between the opioid potentiating properties of intravenous or epidural clonidine for both intra- and postoperative analgesia.<sup>63,64</sup>

In summary, the current results indicate that the tested  $\alpha_2$  agonists can potentiate the analgesic properties of opioids but have no intrinsic antinociceptive effects on spinal reflexes on their own. The potentiating activities of the  $\alpha_2$  agonists could be measured both in terms of a reduction of the amount of opioid needed to reach a particular level of analgesia and in terms of a longer duration of analgesia with a fixed dose of the opioid. The potentiations were observed with various  $\alpha_2$  agonists and opioids and appeared independent of the routes of administration. With regard to the most optimal  $\alpha_2$  agonist to be used for potentiation of opioid analgesia and its possible mechanism of action, more experimental work is needed. Especially compounds acting selectively at one of the  $\alpha_2$ -adrenoceptor subtypes may help to clarify the interactions between the noradrenergic- and opiate system.

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