Antinociceptive Activity of the Novel Fentanyl Analogue iso-Carfentanil in Rats

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ABSTRACT—A large number of fentanyl analogues have been synthesized so far, both to establish the structure-activity-relationship (SAR) and to find novel, clinically useful antinociceptive drugs. In this study, the newly synthesized fentanyl analogue 3-carbomethoxy fentanyl (iso-carfentanil) was compared to fentanyl for its antinociceptive activity (tail-immersion test) in rats. It was revealed that the introduction of a 3-carbomethoxy group in the piperidine ring of fentanyl skeleton reduced the potency and shortened the duration of action of the parent compound, i.e., fentanyl. The antinociceptive potency of 3-carbomethoxy fentanyl is influenced mainly by the steric factor (voluminosity of the carbomethoxy group and the cis/trans isomerism), while the chemical nature of the group is probably irrelevant. This is in agreement with SAR studies of other 3-substituted fentanyl analogues. In contrast to potency, the duration of action is not affected by cis/trans isomerism. It is assumed that the time course of action of 3-carbomethoxy fentanyl is influenced by the nature of the carbomethoxy group. Since the potency and the duration of action of this novel antinociceptive compound are interesting from the aspect of SAR studies and have potential promise for clinical application, 3-carbomethoxy fentanyl deserves to be extensively evaluated.

Keywords: Antinociception, Fentanyl, Carfentanil, Structure-activity-relationship

Fentanyl (Fig. 1), the prototype of the 4-anilidopiperidine class of synthetic opioid analgesics, is widely and successfully used to supplement general anesthesia or to treat postoperative and cancer pain (1–3). However, like other μ-agonists, fentanyl produces serious adverse effects including respiratory depression, muscle rigidity and on prolonged use, tolerance and addiction. In order to discover an analgesic with an improved pharmacodynamic and pharmacokinetic profile, extensive efforts during last four decades have been devoted to syntheses of a large number of fentanyl analogues and establishing the structure-activity-relationship (SAR) of the 4-anilidopiperidine class of analgesics (4–8). As a result of such efforts, several congeners of fentanyl, alfentanil, sufentanil and remifentanil were discovered and have found clinically utility as anesthesia adjuncts (9–12). Several other compounds are still under extensive evaluation in animals nowadays, while some of them are proposed as a useful tools for studying the opioid receptors (13–17). The availability of this range of synthetic opioids and better understanding of the relationship between their structure and the pharmacokinetic and the pharmacodynamic properties provide greater flexibility and increase safety in the management of pain and stress responses during surgery. It has been previously demonstrated that antinociceptive potency of the 3-alkyl fentanyl analogues is predominantly influenced by the steric factor; i.e., the voluminosity of the alkyl group and the cis/trans isomerism (4, 18). For example, the presence of the methyl group on the piperidine ring in the fentanyl skeleton results in about a tenfold increase in antinociceptive potency, while more voluminous alkyl groups cause a gradual drop in the activity compared to fentanyl itself (4, 18–21). Also, the analogues with 3-alkyl groups in the cis position are known to be more potent than the trans isomers (4, 18, 20).

It has already been reported that fentanyl analogues with relatively small polar groups, such as carbomethoxy or methoxyethyl in position 4 of the piperidine ring, were far more active than the parent compound (22). Thus,
carfentanil (4-carboxymethoxy fentanyl) (Fig. 1) is about thirty times more potent than fentanyl and has been used for immobilization of wild animals (22–24). Lofentanil (the levorotatory form of the cis 3-methoxy analogue of carfentanil) is approximately five times more potent than fentanyl, with a remarkable long duration of action; however, it does not have clinical significance (25). Contrary to this, sufentanil is of great clinical value due to its high potency, short duration of action and high safety margin (1, 2, 9).

The objective of SAR studies is to approach the ideal analgesic profile, focusing mainly on potency, safety and duration of action. In view of the interesting SAR that was obtained with 3-alkyl fentanyl analogues (4, 18), as well as analogues substituted at C-4 by a carboxymethoxy group (22–25), 3-carboxymethoxy fentanyl (iso-carfentanil) (Fig. 1) was synthesized (26) and evaluated for analgesic activity in rats.

**MATERIALS AND METHODS**

Wistar rats of either sex (200–250 g) were used. Prior to each experiment, the animals were habituated to the handling and experimental procedure for three consecutive days. The antinociceptive activity was determined by the tail-immersion test (27). 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 ± 0.5°C) and the time for tail-withdrawal was measured as a response latency. To minimize tissue damage by repeated testing, a 10-s cutoff time was imposed for all animals that failed to respond to the stimulus (28). This means that the maximal duration of a single exposure of rat tail to hot water was 10 s. Predrug response latency was obtained 5 min before i.p. drug administration. Postdrug response latency was measured after i.p. administration of test compound at 5, 10, 15, 20, etc. min. Response latency is expressed as a percent maximum possible effect (%MPE) and calculated according to the following formula: %MPE = (postdrug latency – predrug latency) / (cutoff time – predrug latency) × 100.

Dose-response curves were analyzed by linear regression. The ED$_{50}$ and 95% confidence limits were estimated from the dose-response curve by using standard statistical software (29).

Fentanyl citrate (ICN Yugoslavia, Belgrade, Yugoslavia) and (±)cis and (±)trans 3-carboxymethoxy fentanyl oxalate were dissolved in saline and injected i.p. in a final volume

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Fig. 1. Chemical structures of fentanyl, carfentanil, (±)cis 3-carboxymethoxy fentanyl and (±)trans 3-carboxymethoxy fentanyl.

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The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institute of Health (NIH Publication No. 85–23, revised 1985).
of 2 ml/kg (26). Naloxone hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) was also dissolved in saline and injected s.c. in the back before the i.p. injection of the test compound in the same volume. The order of injections was established on the basis of the time-effect curve and allowed testing antinociception at the time of peak effects of both s.c. naloxone and the i.p. test compound (Fig. 2). Although, naloxone expressed a significant antagonism over the range of 15–25 min after injection, the peak effect of naloxone occurred 20 min after its s.c. injection mostly (not shown). This result is in accordance with the previous finding (30). Since we have found that the antinociceptive effects of ED₉₀ of intraperitoneal fentanyl, (±)cìs and (±)trans 3-carbomethoxy fentanyl peaked at about 10, 10 and 5 min, respectively (Fig. 3: A, B and C), and the peak effect of naloxone occurred 20 min after its s.c. injection (not shown), naloxone was applied: 10, 10 and 15 min before the i.p. injection of fentanyl, (±)cìs and (±)trans 3-carbomethoxy fentanyl, respectively (Fig. 2). Control animals received the corresponding volume of saline (s.c.) instead of naloxone plus test compound. The percent inhibition of responding effects by the naloxone pretreatment was expressed as follows: Percent inhibition of antinociception = 100 – [MPE (in the presence of naloxone) / MPE (in the presence of saline)] × 100 (31).

Dose-response curves were analyzed by linear regression. ID₉₀ (the dose of naloxone that caused a 50% inhibition of antinociception) with 95% confidence limits were then calculated and compared (29). To compare time courses of the effects produced by the several drugs, data were expressed as the area under the curve (AUC); e.g., the area of a series of trapezoids in which the height was postdrug response latency minus the predrug response latency (s) and the base, the interval (min) between measurements. This provided an index with the dimension of second-minute (32). The slopes of the regression lines were com-

Fig. 3. The time- (A, B and C) and log dose- (D) response curves on the tail immersion for fentanyl (A and D), (±)cìs 3-carbomethoxy fentanyl (B and D) and (±)trans 3-carbomethoxy fentanyl (C and D) given i.p. in rats. A: fentanyl (0.0073 mg/kg, ●; 0.011 mg/kg, ▲; 0.016 mg/kg, ▼; 0.030 mg/kg, ■); B: (±)cìs 3-carbomethoxy fentanyl (0.016 mg/kg, ●; 0.023 mg/kg, ▲; 0.045 mg/kg, ▼; 0.073 mg/kg, ■); C: (±trans 3-carbomethoxy fentanyl (0.08 mg/kg, ●; 0.10 mg/kg, ▲; 0.17 mg/kg, ▼; 0.29 mg/kg, ■); D: fentanyl (0.0073–0.016 mg/kg, ▲), (±)cìs 3-carbomethoxy fentanyl (0.016–0.045 mg/kg, ●) and (±)trans 3-carbomethoxy fentanyl (0.08–0.17 mg/kg, ■). MPE = maximum possible effect. Each point represents the mean ± S.E.M. of the antinociception in six to eight rats.
pared by using a test for parallelism (29).

Both (+)cis and (+)trans 3-carbomethoxy fentanyl were examined as a racemic mixture. Doses of the drugs were calculated for the free base. To test whether saline injection in control rats has any effect on the tail immersion latency, the t-test for paired values was used (29). A P value of less than 0.05 was considered statistically significant. Relative potency estimates were considered statistically significant when 95% confidence limits did not overlap 1.0.

RESULTS

The i.p. injection of fentanyl, (+)cis and (+)trans 3-carbomethoxy fentanyl produced a dose-dependent increase in latency of the tail-immersion response generated by high-intensity thermal stimuli (Fig. 3: A, B and C). Although all three tested compounds displayed linear dose-response functions, the slopes of (+)cis and (+)trans 3-carbomethoxy fentanyl were significantly reduced (P<0.05) in comparison with fentanyl (Table 1, Fig. 3D). Based on the ED₅₀ values, the relative order of potency was as follows: fentanyl (1) ≥ (+)cis 3-carbomethoxy fentanyl (0.43) > (+)trans 3-carbomethoxy fentanyl (0.095) (Table 1).

Maximal antinociceptive responses were obtained 5—10 min after i.p. injection of both isomers of 3-carbomethoxy fentanyl and 10—15 min after i.p. injection of fentanyl (Fig. 3: A, B and C). The duration of the effect produced by all tested compounds was dose-dependent (Fig. 3: A, B and C). Because doses of the tested compounds given i.p. that produced the same magnitude of response appeared to have different time courses (Fig. 3: A, B and C), we examined the relationship between the MPE produced by a given i.p. dose of a drug and AUC for the associated effect (Table 1). The rank order of the magnitude of the slope of the AUC-MPE plot was: (+)cis 3-carbomethoxy fentanyl (2.3) ≥ (+)trans 3-carbomethoxy fentanyl (2.7) < fentanyl (4.1). This indicated that for doses producing comparable effects, (+)cis and (+)trans 3-carbomethoxy fentanyl displayed significantly (P<0.05) shorter time-courses than fentanyl. Expressed in min, fentanyl, (+)cis and (+)trans 3-carbomethoxy fentanyl, exhibited duration of antinociception of 60, 30 and 30 min, respectively (time that is necessary for the tail withdrawal response to reduce to 50% MPE after i.p. injection of equi-effective doses (ED₅₀) of three tested compounds) (Fig. 3: A, B and C).

 Pretreatment of the rats with s.c. naloxone produced a dose-dependent inhibition of the antinociceptive effects produced by the i.p. injection of a maximally effective dose (ED₅₀) of fentanyl (0.03 mg/kg), (+)cis 3-carbomethoxy fentanyl (0.073 mg/kg) and (+)trans 3-carbomethoxy fentanyl (0.29 mg/kg) (Fig. 4: A, B, C and D). The ID₅₀s of the three tested compounds were not statistically different (P>0.05) (Table 2).

In control experiments, i.p. injection of saline had no effect on the animal’s tail immersion latency (P>0.05); the latencies before and after saline injection were found to be 2.32 ± 0.32 and 2.40 ± 0.25 s, respectively (n = 8) (not shown).

DISCUSSION

In this study, the newly synthesized fentanyl analogues, (+)cis and (+)trans 3-carbomethoxy fentanyl (26) were examined for their antinociceptive activity and compared to fentanyl by the tail-immersion test. It was found that the introduction of a carbomethoxy group in position 3 of the piperidine ring of fentanyl decreased antinociceptive activity by a factor of 2 and 10 in (+)cis and (+)trans 3-carbomethoxy fentanyl, respectively. In view of the previous

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<th>Table 1. Summary of MPE dose-response curves and AUC-MPE curves for i.p. fentanyl, (+)cis 3-carbomethoxy fentanyl and (+)trans 3-carbomethoxy fentanyl in rats</th>
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<tr>
<td>Fentanyl (0.0073 — 0.016 mg/kg)</td>
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<td>MPE dose responses</td>
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<td>ED₅₀ (mg/kg)</td>
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<td>AUC-MPE response</td>
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1Results are summarized from data presented in Fig. 3D. 2ED₅₀ were calculated from three doses of each compound with 6 — 8 rats per dose. n = total number of animals employed to produce the respective dose-response curve. CL = confidence limits, r = correlation coefficient, MPE = maximum possible antinociceptive effect, AUC-MPE = area under the curve-maximum possible antinociceptive effect. *Significantly (P<0.05) different in comparison with fentanyl.
Fig. 4. The time- (A, B and C) and log dose- (D) response curves of the antagonism by s.c. naloxone (0.00156–0.05 mg/kg) of the antinociceptive effects of i.p. fentanyl (0.03 mg/kg, A and D), (±)cis 3-carboxymethoxy fentanyl (0.073 mg/kg, B and D) and (±)trans 3-carboxymethoxy fentanyl (0.29 mg/kg, C and D) in rats. A, B and C: naloxone (0.05 mg/kg, ▲; 0.025 mg/kg, ▼; 0.0125 mg/kg, ■; 0.00625 mg/kg, ●; 0.00156 mg/kg, ○) and saline (▽). D: fentanyl (0.03 mg/kg, □), (±)cis 3-carboxymethoxy fentanyl (0.073 mg/kg, ○) and (±)trans 3-carboxymethoxy fentanyl (0.29 mg/kg, ○). MPE = maximum possible effect (antinociception). Each point represents the mean ± S.E.M. of the effects obtained in six to eight rats.

Table 2. Summary of ID50 and slope values for the dose-dependent antagonism by s.c. naloxone of the antinociceptive effects of i.p. fentanyl, (±)cis 3-carboxymethoxy fentanyl and (±)trans 3-carboxymethoxy fentanyl in rats

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<th>Fentanyl (0.03 mg/kg, i.p.)</th>
<th>(±)cis 3-Carboxymethoxy fentanyl (0.073 mg/kg, i.p.)</th>
<th>(±)trans 3-Carboxymethoxy fentanyl (0.29 mg/kg, i.p.)</th>
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<td>(n = 30)</td>
<td>(n = 20)</td>
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<td>ID50 (mg/kg)</td>
<td>0.008</td>
<td>0.004</td>
<td>0.016</td>
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<tr>
<td>95% CL</td>
<td>(0.005 – 0.013)</td>
<td>(0.001 – 0.016)</td>
<td>(0.011 – 0.022)</td>
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<td>slope</td>
<td>35 ± 3.5</td>
<td>30.1 ± 3.7</td>
<td>38.6 ± 1.8*</td>
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<td>r</td>
<td>0.990</td>
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*Results are summarized from data presented in Fig. 4D. The lowest dose of compound given i.p. that produces the maximum possible antinociceptive effect = ED50 (see Fig. 3A, B and C for the antinociceptive effects of the respective doses). n = number of animals required to produce the naloxone antagonism curve. Each naloxone antagonism curve was constructed from at least three doses of naloxone with 6 – 8 rats per naloxone dose. *Significantly (P<0.05) different in comparison with fentanyl and (±)cis 3-carboxymethoxy fentanyl.
findings of Van Bever et al., as well as the results of our preliminary investigation of a series of newly synthesized 3-alkyl fentanyl analogues, it is evident that 3-carbomethoxy fentanyl is far less active than 3-methyl and 3-ethyl fentanyl (18, 20). Otherwise, it seems that (±)cis 3-carboxemethoxy fentanyl is equipotent to the (±)cis 3-propyl fentanyl and exceeds the potency of (±)cis 3-allyl fentanyl, (±)cis 3-butyl fentanyl, as well as (±)cis 3-benzyl fentanyl (4, 18, 21). Based on the study of SAR of 3-alkyl fentanyl analogues, it has been previously concluded that the antinociceptive activity of 3-alkyl fentanyl analogues depends on the voluminosity of the alkyl group and the cis/trans isomerism (4, 18). The difference in the activity between (±)cis and (±)trans 3-carbomethoxy fentanyl, observed in this study, parallels such examples, since the cis isomer is about 4 times more active than the trans one (Table 1). In addition, since no functionality is present in the case of 3-propyl fentanyl, the observed similarity in antinociceptive potency would result exclusively from the similar voluminosity of the 3-propyl and 3-carbomethoxy group. Consequently, it seems that a steric factor had a predominant role in the antinociceptive potency of 3-substituted fentanyl analogues, while the nature of the substituent was probably irrelevant. Furthermore, based on the available information on the pharmacological properties of 4-carbomethoxy fentanyl, it is evident that (±)cis 3-carbomethoxy fentanyl is considerably (about 60 times) less potent in comparison with its regioisomer, carfentanyl (22). Here again, the influence of steric factor is confirmed, since there is no difference in functional groups between 3-carbomethoxy and 4-carbomethoxy fentanyl. Therefore, the main conclusion in this research was that the antinociceptive potency of 3-carbomethoxy fentanyl is independent of the nature of the substituent group, i.e., it is influenced by the steric factor only. Nevertheless, it is still possible that the reduced potency of 3-carbomethoxy fentanyl is a result of altered pharmacokinetic properties.

On the other hand, it is evident that 3-carbomethoxy group provides more rapid onset and shorter duration of action. Because the length of surgical procedures is often unpredictable and because the level of surgical stimulation against which the depth of anesthesia must be balanced is highly variable, the advantages of predictably short-acting alfentanil and remifentanil in comparison with fentanyl have already been shown (12, 33). In addition, their rapid onset of action allows fast curtailment of an evoked cardiovascular stress response during anesthesia for surgery (12, 33). Therefore, the rapid onset and offset of effects of opioid analgesics are preferable characteristics in anesthesia that enable precise tailoring of analgesia to the prevailing surgical conditions. At the doses producing comparable changes in the antinociceptive response measures, (±)cis and (±)trans 3-carbomethoxy fentanyl produced effects that were substantially shorter as compared with the effects produced by fentanyl. In contrast to potency, the time course of action is not significantly influenced by stereoisomery; i.e., cis or trans. At present, we can only speculate that the difference in the time of onset, as well as duration of action after i.p. administration of all tested compounds, probably results from the differences in their physiochemical characteristics and/or metabolism. It has already been observed that the more hydrophilic or ionized substituents lead to analgesics with less lipid solubility (lower partition coefficients), little or no accumulation in fat tissues, and rapid excretion (33). Therefore, in contrast to potency, the influence of chemical nature of the carbomethoxy group upon the onset and the duration of antinociceptive activity is more probable. For example, shorter action of both isomers of 3-carbomethoxy fentanyl could also be due to susceptibility of the carbomethoxy group to rapid hydrolysis by non-specific esterases, such as is the case with the ultra-short-acting fentanyl analogue remifentanil (10). To test this hypothesis, further pharmacological investigation is necessary. In addition, drug-receptor interaction could also have an influence on the onset and duration of effects, as it has already been shown in the case of alfentanil (11), buprenorphine (34) or (+)-cis 3-methyl fentanyl (35).

In this study, time-response curves for antinociception were determined for each test compound in the absence and in the presence of increasing doses of naloxone (Fig. 4: A, B and C). Since both (±)cis and (±)trans 3-carbomethoxy fentanyl were sensitive to naloxone antagonism, it was concluded that the antinociceptive effects of 3-carbomethoxy fentanyl is opioid-receptor-mediated.

In accordance with the experiments done using fentanyl i.p. in rats (36, 37) the side effects of (±)cis 3-carbomethoxy fentanyl and (±)trans 3-carbomethoxy fentanyl observed in our study were morphine-like; i.e., characterized by stiffness of the tail, catalepsy, loss of righting reflex, loss of the pinna reflex, etc. Doses that are 2–3 times higher than those required to produce complete block on the tail-immersion test were commonly associated with a loss of pinna reflex and tail stiffness, while much higher doses produced a significant increase in the incidence of catalepsy and loss of righting reflex. It should be stressed, however, that the doses of all three compounds needed to produce maximal antinociception did not have any significant effect on motor the function, nor was any death observed during the following 7 post-treatment days.

The slope of the dose-response curve for antinociception of 3-carbomethoxy fentanyl in the tail-immersion test at 55°C was linear and steep, although shallower compared to fentanyl (38). This finding taken together with: 1) considerable potency in thermal test using high intensity of stimuli (28, 39, 40), 2) fentanyl-like profile of behavior effects (36), and 3) great sensitivity to antagonism by naloxone (2) im-
plies that 3-carbomethoxy fentanyl produces effects in a similar manner as fentanyl, most probably through the action on μ-opioid receptors. To test this hypothesis, further work by using selective antagonists of opioid receptors is required.

Since the antinociceptive activity of the 4-anilidopiperidine analogues is usually highly dependent on stereochemistry, it is probable that one enantiomer of both tested compounds will be more active than the tested racemic mixture.

The synthesis and the pharmacological evaluation of 3-carboxyethoxy fentanyl will provide better understanding of SAR of 3-substituted fentanyl analogues. Based on the results obtained in these studies, it is concluded that the steric factor has a predominant role in the antinociceptive potency of 3-substituted fentanyl analogues, while the nature of the substituent is probably irrelevant. In addition, it was revealed that 3-carboxyethoxy fentanyl is not especially potent, neither selective in comparison with fentanyl, but is notable for its short duration of action. Unfortunately, so far pharmacokinetics of 3-carboxyethoxy fentanyl have not been evaluated, so the closer SAR could not be determined. Nevertheless, in view of the present findings, this novel antinociceptive compound appears to be worthy of further study.

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