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NEUROTOXICITY EVALUATION OF FENTANYL ANALOGS IN RATS

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This study aimed at evaluating the neurotoxicity of fentanyl analogs: (±)-cis-3-carbomethoxy fentanyl (C) and (±)-trans-3-carbomethoxy fentanyl (T) in rats. C and T are less potent (2.4-3.1 and 8.4-12.3 times, respectively) than fentanyl (F) in producing both antinociception and morphine-like neurotoxic effects: loss of pinna reflex, Straub tail, impairment of motor coordination, catalepsy, loss of corneal reflex and loss of righting reflex. All of the effects tested were dose-dependent and they were abolished by pretreatment with naloxone, nonselective antagonist of opioid receptors, indicating that they are mediated via opioid receptors. Further, F, C and T exhibited similar relative potencies in producing all tested effects, indicating that similar receptors are involved in producing antinociceptive and neurotoxic effects, most probably of μ type. By using equi-antinociceptive doses, C and T produced significantly shorter duration of both antinociception and neurotoxicity than F. No significant differences between therapeutic indices for F, C and T were found, indicating that these compounds are equally safe and tolerable in respect to the neurotoxic effects tested. Neurotoxicity testing presented in this paper may be useful in studying the structure-activity relationship of opioid congeners.

Key words: analog, fentanyl, neurotoxicity, rats

INTRODUCTION

Fentanyl (Fig. 1A) belongs to 4-anilidopiperidine class of synthetic opioid analgesics (Janssen *et al*., 1996). It is a strong opioid analgesic with widespread use in the treatment of moderate to severe pain. However, the clinical use of the fentanyl is limited by serious central nervous system side effects such as respiratory depression, sedation, nausea, muscle rigidity, and after prolonged use, tolerance and addiction (Geppetti and Benemei, 2009). Like morphine and most other currently available strong opioid analgesics, fentanyl exerts analgesic and adverse effects primarily through the opioid μ receptors (Schumacher *et al*., 2007).

The most common approach in searching for novel drugs is structural modification of the well known compounds (Mićović *et al*., 2000; Ivanović *et al*., 2004a; Ivanović *et al*., 2004b; Vučković *et al*., 2009). Among the important properties of the opioids that can be altered by structural modification are their affinities for various types of opioid receptors, activities as agonists versus antagonists, lipid solubilities, and their susceptibility/resistance to metabolic breakdown (Feldman *et al*., 1991; Scholz *et al*., 1996; Ananthan, 2006).

Carfentanil (Fig. 1B) is 20-30 times more potent than fentanyl and is used in veterinary medicine for immobilization of wild animals (Van Daele, 1976; De Vos, 1978). In previous work (Mićović *et al.*, 1998; Vučković *et al*., 2000), the regioisomer of carfentanyl, 3-carbomethoxy fentanyl (Fig. 1C and 1D), or "iso-carfentanil" was prepared and tested for analgesic activity in rats. It was found that (±)-cis-3-carbomethoxy fentanyl (Fig. 1C) and (±)-trans-3-carbomethoxy fentanyl (Fig. 1D) were about 2 and 10 times less potent than fentanyl, respectively, but their tolerability and safety compared with fentanyl remained unexplored. This study is aimed at evaluating the relative tolerability and safety of (±)-cis and (±)-trans-3-carbomethoxy fentanyl, by using tests for assesing morphine-like neurotoxicity in rats, such as loss of pinna reflex, impairment of motor coordination, Straub tail (tail in an erect position), catalepsy (muscular rigidity and immobility), loss of corneal reflex and loss of righting reflex. In addition, in regard to the neurotoxic effects, structure-activity relationship (SAR) of (±)-cis and (±)-trans-3-carbomethoxy fentanyl was to be established.

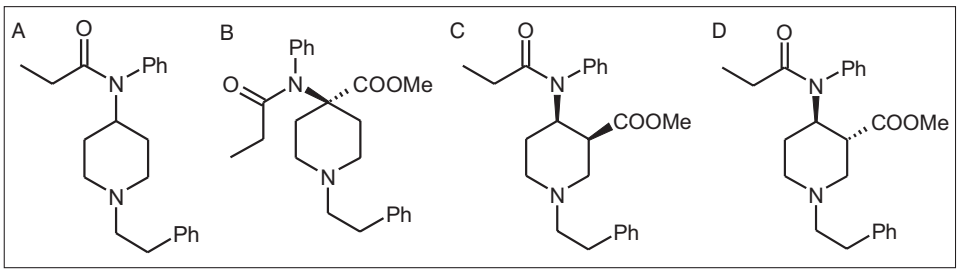


Figure 1. Fentanyl (A), carfentanil (B), (±)-cis-3-carbomethoxy fentanyl (C) and

MATERIALS AND METHODS

Animals

Male Wistar rats (200–250 g) obtained from Military Farm (Belgrade, Serbia) were used. All experiments were approved by the Institutional Animal Ethics Committee which operates in accordance with Revised Guide for the Care and Use of Laboratory Animals (NIH Guide, Volume 25, Number 28, 1996). The animals were housed in groups of 4 in plexiglass cages (36.5 x 21 x 14 cm) under standard conditions: temperature of 22°C ± 1°C, and a 12/12 h light/dark cycle with lights on at 08.00 h. Food pallets and tap water were available *ad libitum*, except during the experimental procedure. Prior to each experiment the animals

were habituated to handling and experimental procedures for at least three consecutive days. Experiments were done in a sound–proofed, diffusely illuminated room maintained at a temperature of 22±1°C. They were performed at the same time of the day between 9:00 and 13:00 h to avoid diurnal variation in behavioral tests. The animals were unrestrained during all experimental procedures, except antinociception testing. Experimental groups consisted of 6–8 rats. Each animal was tested only once and was killed with an intraperitoneal injection of sodium thiopental.

Antinociception testing

In the first set of experiments, antinociceptive activity was determined by tail-immersion test (Janssen *et al*., 1963). In brief, the rat was placed in a hemicylindrical plexiglass cage 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. In order to minimize tissue damage by repeated testing, a cut-off time of 6 s was adopted. This means that the maximal duration of a single exposure of rat tail to hot water was 6 sec. Predrug response latency was obtained 5 min before i.p. drug (or saline solution in the control group) administration. Postdrug response latency was measured after intraperitoneal (i.p.) administration of test compound (or saline solution in the control group) at 5, 10, 15, 20, 40, 60, 90 etc. min. The data are expressed quantally as the number of animals in which the antinociception was observed versus total number of animals receiving the same treatment. For antinociception, the following criterion was used: an antinociceptive effect was said to have occurred if postdrug response latency was ≥ 6s.

Neurotoxicity testing

In the second set of experiments, toxic effects were tested in the following order: loss of pinna reflex, loss of corneal reflex, tail stiffness (Straub tail), catalepsy, impairment of motor coordination and loss of righting reflex in each single rat. Experimental groups consisted of 6–8 rats. The data are expressed quantally as the number of animals in which the effect was observed versus total number of animals receiving the same treatment.

Testing was performed once before and at 5, 20, 40, 60, 90 etc. min after i.p. drug (or saline solution in the control group) injection by two observers unaware of the pharmacological treatment. Tail stiffness ("Straub tail") was assessed by observation, as well as touching the tail (Benthuysen *et al*., 1986). The pinna reflex was tested by touching the pinna with a pencil tip. (Meert *et al*., 1988). The corneal reflex was tested by touching the cornea with a small peace of cotton (Meert *et al*., 1988). Catalepsy was defined as the failure of the animal to move within 60 s from a position in which the forepaws and hind paws were placed on bars 10 cm from the floor (Klemm, 1989; Vučković *et al*., 1998). The righting reflex was measured by placing the animal onto its back and measuring how long it took to regain an upright position. The righting reflex was considered absent when all four limbs remained off the table surface for at least 30 s (Yang *et al*., 1992; Ivanović *et al*., 1995). Impairment of motor coordination was defined as the inability of the rat to

descend in a coordinated fashion a 60-degree-inclined wire mesh ramp (Yaksh *et al*., 1986).

Drugs Administration

Fentanyl citrate (ICN Yugoslavia, Belgrade, Serbia) and (±)-cis and (±)-trans-3-carbomethoxy fentanyl oxalate were dissolved in saline and injected intraperitoneally at a final volume of 2 mL/kg. Both (±)-cis and (±)-trans-3-carbomethoxy fentanyl (Faculty of Chemistry, University of Belgrade, Serbia) were examined as a racemic mixture. Doses of the drugs were calculated for the free base. Naloxone hydrochloride (Sigma Chemical Co. St. Louis, USA) was also dissolved in saline, and injected subcutaneously (s.c., 1 mg/kg) in the back 10 min before the intraperitoneal (i.p.) injection of the test compound in the same volume. In order to test whether saline injection has any effect on nociception or toxic behavior, 2 mL/kg of saline were administrated i.p. in a control group of rats.

Statistical Analysis

To permit direct comparison of different compounds and different effects, basic data for each animal were transformed to a quantal response (presence or absence of expected drug effect). For each effect and each dose maximum response obtained during time of measurement was used for evaluation. Then, computations were done according to the methods of Tallarida and Murray (1986).

RESULTS

Fentanyl (F; 0.0073-0.120 mg/kg; ip), (±)cis 3-carbomethoxy fentanyl (C; 0.016-0.326 mg/kg; ip) and (±)trans 3-carbomethoxy fentanyl (T; 0.08-1.22 mg/kg; i.p) produced dose-dependent increase in antinociception, loss of pinna reflex, impairment of motor coordination, Straub tail, catalepsy, loss of corneal reflex and loss of righting reflex (Fig. 2). For each effect tested, probit slopes for F, C and T are not significantly different ($p>0.05$, test for parallelism).

The median effective doses for antinociception (AD_{50}) and toxic effects (TD_{50}), and the relative potencies for F, C and T are presented in Table 1. The median effective doses (ED_{50}) for C and T are significantly higher ($p<0.05$) in comparison with the corresponding doses for F (Table 1), indicating that C and T are less potent than F in producing antinociceptive and toxic effects. Also, T was significantly less potent ($p<0.05$) in producing all observed effects compared with C (Table 1). The potency ratios indicate that C (ED_{50}=0.024 (0.017-0.034)) and T (ED_{50}=0.084 (0.054-0.131)) are less potent analgesics (2.4 and 8.4 times, respectively), in comparison with F (ED_{50}=0.010 (0.007-0.014)). In regard to toxic effects, C and T are less potent (2.4-3.1 and 10.8-12.3 times, respectively) than F (Table 1). T is 3.5 and 3.6-5.1 times less potent than C in inducing antinociception and toxicity, respectively (not shown).

Each of tested compounds, exhibited similar ($p>0.05$) relative potencies in producing all tested effects (95% confidence intervals overlap) (Table 1).

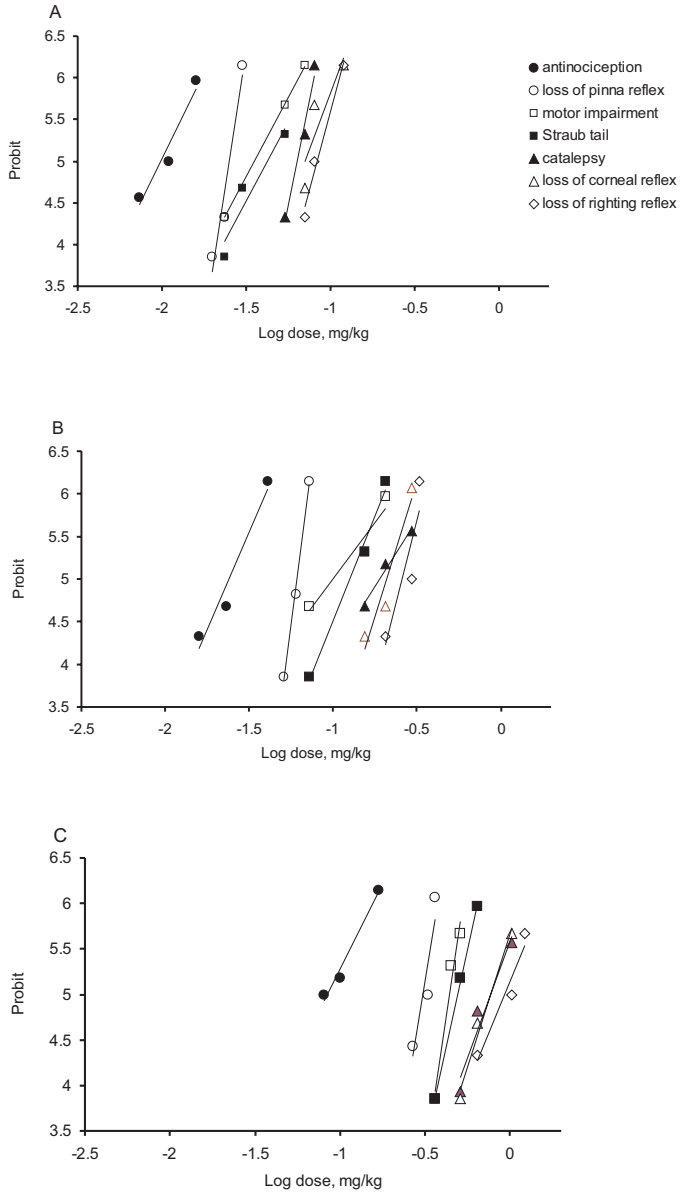


Figure 2. Log dose-probit curves for antinociception (tail-immersion test) and toxic effects for fentanyl (A), (±)-cis-3-carbomethoxy fentanyl (B) and (±)-trans-3-carbomethoxy fentanyl (C) in rats. Doses of the compound tested are expressed in mg/kg. For each effect and each dose, the percentage of rats that respond to treatment is transformed to a probit value. Each point represents the results obtained from 6-8 rats

Table 1. Median effective doses (AD_{50} and TD_{50}), correlation coefficient (r), probit slopes and relative potencies with 95% confidence limits (95% CL) for fentanyl, (±)-cis-3-carbomethoxy fentanyl and (±)-trans-3-carbomethoxy fentanyl in inducing antinociception, loss of pinna reflex, motor impairement, Straub tail, catalepsy, loss of corneal reflex, and loss of righting reflex in rats

Effect	AD_{50} or TD_{50} (95% CL)	r	Probit slope (95% CL)	Relative potency (95% CL)
Fentanyl (0,0073-0,12 mg/kg)				
Antinocicep.[a]	0.010 (0.007-0.014)	0.968	4.08 (-9.3-17.5)	1
Loss of pinna r.	0.025 (0.021-0.030)	0.981	13.1 (-20.1-46.4)	1
Motor impair.	0.036 (0.023-0.054)	1.000	3.8 (3.0-4.5)	1
Straub tail	0.043 (0.030-0.060)	0.957	3.8 (-10.1-18.6)	1
Catalepsy[b]	0.064 (0.054-0.074)	0.983	10.3 (-13.8-34.3)	1
Loss of corn. r.	0.071 (0.053-0.095)	0.871	5.4 (-33.3-44.1)	1
Loss of right. r.[c]	0.084 (0.068-0.100)	0.987	7.5 (-8.3-23.3)	1
(±)-cis-3-Carbomethoxy fentanyl (0.016-0.326 mg/kg)				
Antinocicep.	0.024 (0.017-0.034)	0.977	4.6 (-8.1-17.3)	0.408* (0.247-0.676)
Loss of pinna r.	0.061 (0.052-0.071)	0.999	15.6 (5.3-25.5)	0.412* (0.328-0.518)
Motor impair.	0.099 (0.060-0.166)	0.962	2.7 (-6.9-12.3)	0.357* (0.184-0.693)
Straub tail	0.125 (0.080-0.197)	0.995	5.0 (-1.6-11.6)	0.339* (0.192-0.599)
Catalepsy[b]	0.189 (0.123-0.291)	0.988	3.1 (-3.0-9.3)	0.336* (0.213-0.529)
Loss of corn. r.	0.208 (0.169-0.256)	0.969	6.4 (-14.3-26.9)	0.342* (0.240-0.488)
Loss of right. r[c]	0.257 (0.217-0.303)	0.885	7.7 (-43. 8-59.2)	0.327* (0.250-0.426)
(±)-trans-3-Carbomethoxy fentanyl (0.08-1.22 mg/kg)				
Antinocicep.[a]	0.084 (0.054-0.131)	0.988	3.6 (-3.5-10.7)	0.117* (0.066-0.209)

cont. Table 1.

Loss of pinna r.	0.308 (0.274-0.346)	0.924	11.6 (-49.3-72.4)	0.082* (0.067-0.100)
Motor impair.	0.441 (0.389-0.499)	0.974	12.5 (-24.0-48.9)	0.081* (0.052-0.125)
Straub tail	0.513 (0.404-0.651)	0.982	7.0 (-10.2-24.3)	0.083* (0.055-0.126)
Catalepsy[b]	0.767 (0.587-1.003)	0.971	5.2 (-11.1-21.5)	0.083* (0.061-0.113)
Loss of corn. r.	0.768 (0.587-1.006)	0.991	5.9 (-4.5-16.9)	0.093* (0.062-0.138)
Loss of right. r[c].	0.927 (0.696-1.235)	0.970	4.6 (-10.1-19.2)	0.091* (0.064-0.129)

AD_{50}=median effective doses (ED_{50}) in inducing antinociception. TD_{50} = median effective doses (ED_{50}) in inducing toxic effects. Median effective doses for each effect and each compound was calculated by using 3 doses. One dose is tested in at least 6 rats (Litchfield & Wilcoxon I test). [a]criterion ≥6 s. [b]criterion ≥60 s. [c]criterion ≥30 s.
*Relative potency estimates were considered statistically significant when 95% CL did not overlap 1.0 ($p<0.05$, Litchfield & Wilcoxon II test).

When the doses of all three tested drugs were increased above their antinociceptive doses, the toxic effects appeared in a similar order (Table 1, Fig. 2). Loss of pinna reflex occurred first, followed by impairment of motor coordination and Straub tail. With further increases in doses, catalepsy, loss of corneal reflex and loss of righting reflex occurred (Table 1, Fig. 2).

The therapeutic indices for all three compounds tested (calculated as TD_{50}/AD_{50}) are shown in Table 2. The AD_{50} values for F, C, and T are significantly lower ($p<0.05$) than their TD_{50} values (Table 2). Within each effect, there are no significant differences ($p>0.05$) between TIs for F, C and T (Table 2).

Time course of the antinociceptive and toxic effects obtained with equi-antinociceptive doses ($4xED_{50}$, $8xED_{50}$ and $15xED_{50}$) of F, C and T is presented in Fig. 3. By using equi-antinociceptive doses ($4xED_{50}$ and $8xD_{50}$), C and T produced significantly shorter duration of antinociception than F ($p<0.01$; Mann–Whitney U-test). There is no difference in the duration of analgesia between C and T. Also, C and T exhibited faster onset of analgesia in comparison with F. After i. p. injection of $4xAD_{50}$ of C, T, and F, analgesia peaked at 5, 5 and 15 min, respectively (Figs. 3 A and B).

At doses $15xAD_{50}$, C and T produced significantly ($p<0.05$ or $p<0.01$; Mann–Whitney U-test) shorter duration of loss of pinna reflex, impairment of motor coordination, Straub tail, catalepsy, loss of corneal reflex, and loss of righting reflex, than F (Figs. 3C-H, Table 2). There is no difference in the duration of toxic effects between C and T. In the majority of the effects observed, C and T achieved peak effect faster (at the first time point measurement, *ie* 5 min) than F (at the second time point measurement, *ie* 20 min) (Figs. 3C-H).

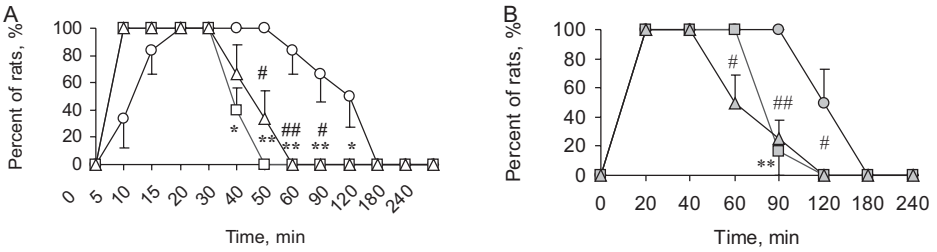
Table 2. Therapeutic indices (TI) with 95% confidence limits (95% CL) and duration of toxic effects (loss of pinna reflex, motor impairement, Straub tail, catalepsy, loss of corneal reflex, loss of righting reflex) for fentanyl, (±)-cis-3-carbomethoxy fentanyl and (±)-trans-3-carbomethoxy fentanyl in rats

Drug tested	Fentanyl		(±)-cis-3-Carbomethoxy fentanyl		(±)-trans-3-Carbomethoxy fentanyl	
Effects	Therapeutic index (95% CL)	Duration (min)[a]	Therapeutic index (95% CL)	Duration (min)[a]	Therapeutic index (95% CL)	Duration (min)[a]
Loss of pinna r.	2.56 * (1.71-3.83)	120	2.53* (1.73-3.70)	60^{+}	3.68* (2.32-5.83)	40^{+}
Motor impair.	3.61* (2.07-6.31)	90	4.12* (2.23-7.65)	40^{+}	5.27* (3.31-8.37)	40^{+}
Straub tail	4.33* (2.62-7.15)	60	5.20* (2.94-9.20)	20^{+}	6.13* (3.69-10.16)	40^{+}
Catalepsy	6.47* (4.34-9.63)	60	7.87* (4.54-13.64)	20^{+}	9.16* (5.44-15.41)	20
Loss of corneal r.	7.26* (4.55-11.57)	60	8.65* (5.79-12.93)	40^{+}	9.18* (5.45-15.46)	20^{+}
Loss of righting r.	8.54* (5.60-13.02)	60	10.67* (7.27-15.65)	20^{+}	11.07* (6.51-18.82)	5^{+}

Therapeutic index (TI) is calculated as TD_{50}/AD_{50} potency ratio for each drug *$p<0.05$, Litchfield & Wilcoxon II test. If 95% CL for a TI fails to include 1.0, then TD_{50} and AD_{50} are significantly different. [a]Duration of action after i.p. injection of equi-analgesic doses of 15 x AD_{50} by using a criteria >50% of rats responding $^{+}$ $p<0.05$ indicates a significant shorter duration of action of fentanyl analog in comparison to fentanyl (Mann–Whitney U-test)

Naloxon hydrochloride (1 mg/kg; sc) given 10 min before ip injection of 8xED_{50} and 15xED_{50} of F, C and T abolished antinociceptive and toxic effects (not shown).

Ip injection of saline (0.2 mL/kg) had no effect on the animal's behavior, as well as 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).



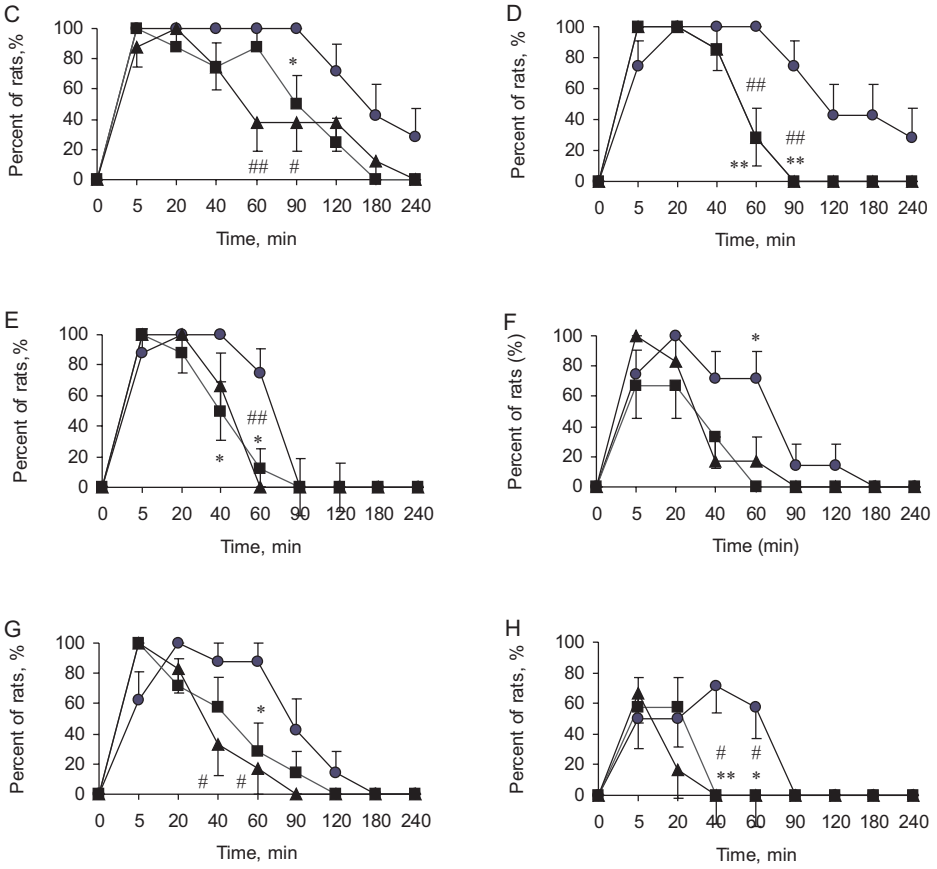


Figure 3. Time-effect curve for the antinociceptive and toxic effects of fentanyl (circle), (±)-cis-3-carbomethoxy fentanyl (quadrant) and (±)-trans-3-carbomethoxy fentanyl (triangle) in rats. The incidence of antinociception (A and B), loss of pinna reflex (C), impairment of motor coordination (D), Straub tail (E), catalepsy (F), loss of corneal reflex (G), and loss of righting reflex (H) are plotted as a function of time after i.p. injection of $4xAD_{50}$ (white symbols), $8xAD_{50}$ (gray symbols) and $15xAD_{50}$ (black symbols) for of fentanyl, (±)-cis-3-carbomethoxy fentanyl and (±)-trans-3-carbomethoxy fentanyl. Each dose was tested by using 6-8 rats. Each point represents the percentage of rats ± S.E. that respond to the treatment. $^{*}p<0.05$ and $^{**}p<0.01$ indicate a significant difference of the responses of (±)-cis-3-carbomethoxy fentanyl in comparison to fentanyl (Mann-Whitney – U test). $^{\#}p<0.05$ and $^{\#\#}p<0.01$ indicate a significant difference of the responses of (±)-trans-3-carbomethoxy fentanyl in comparison to fentanyl (Mann-Whitney -U test)

DISCUSSION

In the present experiments all three compounds tested: fentanyl (F), (±)cis 3-carbomethoxy fentanyl (C) and (±)trans 3-carbomethoxy fentanyl (T), produced a dose-dependent increase in antinociception and morphine-like effects, such as loss of the pinna reflex, Straub tail, impairment of motor coordination, catalepsy, loss of corneal reflex and loss of righting reflex. This finding is in agreement with previous reports on actions of fentanyl in rats (Meert *et al*., 1988; Ivanović *et al*., 1995; Vučković *et al*., 1998; Vučković *et al*., 2000). C and T are less potent (2.4-3.1 and 8.4-12.3 times, respectively) than fentanyl (F) in producing both the antinociception and neurotoxicity. The antinociceptive and toxic effects of F, C and T were abolished by pretreatment with naloxone, which is a nonselective antagonist of opioid receptors, indicating that toxic effects are mediated by opioid receptors. It was revealed that F, C and T exhibited similar relative potencies in producing all tested effects. If a series of related agonists exhibits identical relative potencies in producing distinct effects, it is likely that these effects are mediated by similar or identical receptor molecules (Bourne and Zastrow, 2007). In the case of F, C and T, they are most probably of the μ type. This is consistent with previous reports that μ opioid receptors are involved in the mechanisms of opioid induced antinociception, Straub tail, muscle rigidity, catalepsy and other morphine-like behavioural effects in rats (Negri *et al*., 1992; Nath *et al*., 1994; Chen *et al*., 1996; Piepponen *et al*., 1997).

Most of the currently available opioid analgesics exert their analgesic and adverse effects primarily through the opioid μ receptors. However, individual strong opioids may interact, at least in part, with different opioid receptor subpopulations or modulate μ opioid receptor signaling in different ways (Pasternak, 2004; Lee *et al*., 2007), that may improve tolerability (Ananthan, 2006; Smith, 2008; Spetea *et al*., 2010).

There are no significant differences between therapeutic indices for F, C and T, which means that these compounds are equally tolerable in regard to the observed neurotoxic effects, and the difference between them is in the potency and the time course of action.

By using equi-antinociceptive doses, C and T produced significantly shorter duration of both, antinociception and neurotoxicity, than F. Also, there is no difference in the duration of effects between C and T. One of the possible explanation for the shorter duration of action of 3-carbomethoxy fentanyl in comparison with fentanyl might be the susceptibility of the carbomethoxy group to rapid hydrolysis by non-specific esterases (Feldman *et al*., 1991). It is possible, also that the introduction of 3-carbomethoxy group in the piperidine ring affects duration of action by altering physicochemical properties (Scholz *et al*., 1996).

SUMMARY

In summary, (±)cis 3-carbomethoxy fentanyl and (±)trans 3-carbomethoxy fentanyl are less potent (2.4-3.1 and 8.4-12.3 times, respectively) than fentanyl in producing both antinociception and neurotoxicity in rats. All three compounds are

equally tolerable and safe drugs in respect to neurotoxic effects. Also, F, C and T exhibited similar relative potencies in producing all evaluated effects, and the structure-activity relationship on neurotoxic effects of fentanyl analogs obtained by introducing carbomethoxy group in the position 3 of the piperidine ring, parallels the structure-activity relationship on antinociception. All of these taken together, might suggest that similar receptors are involved in producing both antinociceptive and neurotoxic effects of F, C and T, most probably of μ type.

Animal testing presented in this paper consists of procedures which can be performed easily and in parallel manner providing several useful pharmacological informations regarding efficacy, potency, time course of action, safety and tolerability, and also, could be indicative whether the observed drug effects are mediated by similar or different receptors. Therefore, we recommend it as a useful approach in studying the structure-activity relationship of opioid congeners.

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ISPITIVANJE NEUROTOKSIČNOSTI ANALOGA FENTANILA KOD PACOVA

VUČKOVIĆ SONJA, SAVIĆ VUJOVIĆ KATARINA, IVANOVIĆ M, DOŠEN-MIĆOVIĆ LJILJANA, TODOROVIĆ Z, VUČETIĆ Č, PROSTRAN M i PROSTRAN MILICA

SADRŽAJ

Cilj studije bio je da se ispita neurotoksičnost analoga fentanila: (±)-*cis*-3-karbometoksi fentanila (C) i (±)-*trans*-3-karbometoksi fentanil (T) kod pacova. C je oko 2,4-3,1, a T oko 8,4-12,3 puta manje potentan od fentanila u izazivanju antinocicepcije i morfinu-sličnih neurotoksičnih efekata u koje spadaju: refleks ušne školjke, Straub-ov rep, poremećaj motorne koordinacije, katalepsija, gubitak kornealnog refleksa i gubitak refleksa uspravljanja. Svi ispitivani efekti su dozno-zavisni i bivaju poništeni ako se u pretretmanu primeni nalokson, neselektivni antagonist opioidnih receptora, što ukazuje da se efekti odigravaju posredstvom opioidnih receptora. Dalje, F, C i T ispoljavaju sličnu relativnu jačinu u izazivanju ispitivanih efekata, što ukazuje da su slični receptori uključeni u mehanizam antinocicepcije i neurotoksičnih efekata, i to su najverovatnije μ receptori. Kad se primenjuju ekviantinociceptivne doze, C i T izazivaju značajno kraće i antinociceptivno i neurotoksično dejstvo od F. Nisu dokazane značajne razlike u terapijskim indeksima između F, C i T, što ukazuje da su ovi lekovi jednako bezbedni i podnošljivi kad su u pitanju ispitivani neurotoksični efekti. Ispitivanje neurotoksičnosti prikazano u ovom radu može biti korisno u proučavanju odnosa između strukture i aktivnosti hemijski srodnih opioida.