3-ALKYL FENTANYL ANALOGUES: STRUCTURE-ACTIVITY -RELATIONSHIP STUDY

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Summary

Introduction. Fentanyl belongs to 4-anilidopiperidine class of synthetic opioid analgesics. It is characterized by high potency, rapid onset and short duration of action. 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 analgesic drugs.

Objective. In this study, newly synthesized 3-alkyl fentanyl analogues were examined for analgesic activity and compared with fentanyl.

Methods. Analgesic activity was assessed by tail-immersion test in rats.

Results. The relative potency was: (±)cis-3-methyl fentanyl (8) > (±)*trans*-3-methyl fentanyl (2) ≥ (±)*cis*-3-ethyl fentanyl (1.5) > fentanyl (1) ≥ (±)*trans*-3-ethyl fentanyl (0.9) > (±)*cis*-3-butyl fentanyl (0.064) ≥ (±)*trans*-3-butyl fentanyl (0.035) > (±)*cis*-3-benzyl fentanyl (0.008) ≥ (±)*trans*-3-benzyl fentanyl (0.0055). (±)*Cis*-3-*iso*-propyl fentanyl, and (±)*cis*-3-phenethyl fentanyl are inactive in doses up to 5 mg/kg. The duration of action (ED_{99}) was: (±)*cis*-3-methyl fentanyl (90 min) > (±)*trans*-3-methyl fentanyl (40 min) ≤ (±)*cis*-3-ethyl fentanyl (60 min) ≥ (±)*trans*-3-ethyl fentanyl (40 min) ≤ fentanyl (50 min) = (±)*cis*-3-butyl fentanyl (50 min) = (±)*trans*-3-butyl fentanyl (50 min) = (±)*cis*-3-benzyl fentanyl (50 min) = (±)*trans*-3-benzyl fentanyl (50 min). Symbols > and < denotes $p<0.05$.

Conclusion. It is concluded that the analgesic potency of 3-alkyl fentanyl analogues is influenced by the steric factor (voluminosity of the group at the position 3 of the piperidine ring and the *cis*/*trans* isomerism). Otherwise, with the exception of 3-methyl fentanyl, the duration of action of 3-alkyl fentanyl analogues is not significantly affected by the stereochemistry.

Key words: fentanyl, analogues, structure-activity-relationship (SAR), analgesic activity

INTRODUCTION

Fentanyl, the prototype of the 4-anilidopiperidine class of synthetic opioid analgesics, is widely used to supplement general anesthesia or to treat postoperative and cancer pain [1]. 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 [2 - 9]. Like other μ agonists, all these drugs suffer from serious adverse effects including respiratory depression, muscle rigidity, nausea, sedation, and with prolonged use, tolerance and addiction [1,2,10 - 12]. The objective of SAR studies is to discover compounds with adequate potency, greater selectivity, and with enhanced pharmacokinetic properties in comparison to existing drugs [2]. The development of novel opioids, as research tools, is almost equally important. The establishment of detailed SAR, in combination with conformation analysis of the ligands, is an important approach to studying receptors [13 - 15].

The SAR studies on fentanyl analogues revealed, among other factors, a great influence of the stereochemistry upon the analgesic activity. For example, it was disclosed that a methyl group introduced in position 3 of the piperidine ring (Fig. 1) may dramatically enhance the activity, depending on the relative and the absolute stereochemistry [16, 17]. Thus, (+)-*cis*-3-methyl fentanyl (≈ 19 x fentanyl) is about 100 times more potent than the (–)-enantiomer. The corresponding racemic *trans* isomer is approximately as active as fentanyl [17]. Also, it was revealed that replacing 3-methyl with an allyl or a propyl group significantly reduces overall potency [18]. These facts encouraged the preparation of several novel 3-alkyl fentanyl derivatives (Fig. 1) and the evaluation of the correlation between the structure (including stereochemistry) of the 3-alkyl group and the analgesic activity.

The aim of the present study was to examine the analgesic activity of newly synthesized: 3-ethyl fentanyl, 3-butyl fentanyl, 3-benzyl fentanyl, 3-iso-propyl fentanyl, and 3-phenethyl fentanyl in rats, and to establish SAR of 3-alkyl fentanyl analogues.

METHODS

Male Wistar rats (200–250 g) obtained from Military Farm (Belgrade, Serbia) were used. Prior to the start the experiments, researchers asked for and obtained the permission from Ethics Committee for Animal Research and Welfare of Faculty of Medicine, University of Belgrade (permission N° 5057/2). All experiments were approved by Ethical Council for Protection of Experimental Animals of Ministry of Agriculture, Forestry and Water Management the Republic of Serbia, which operates in accordance with Animal Welfare Law of our country and IASP (International Association for the Study of Pain) Guidelines for the Use of Animals in Research. The animals were housed in groups of 4 in plexiglas 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 the handling and experimental procedures for at least three consecutive days. Experiments we-

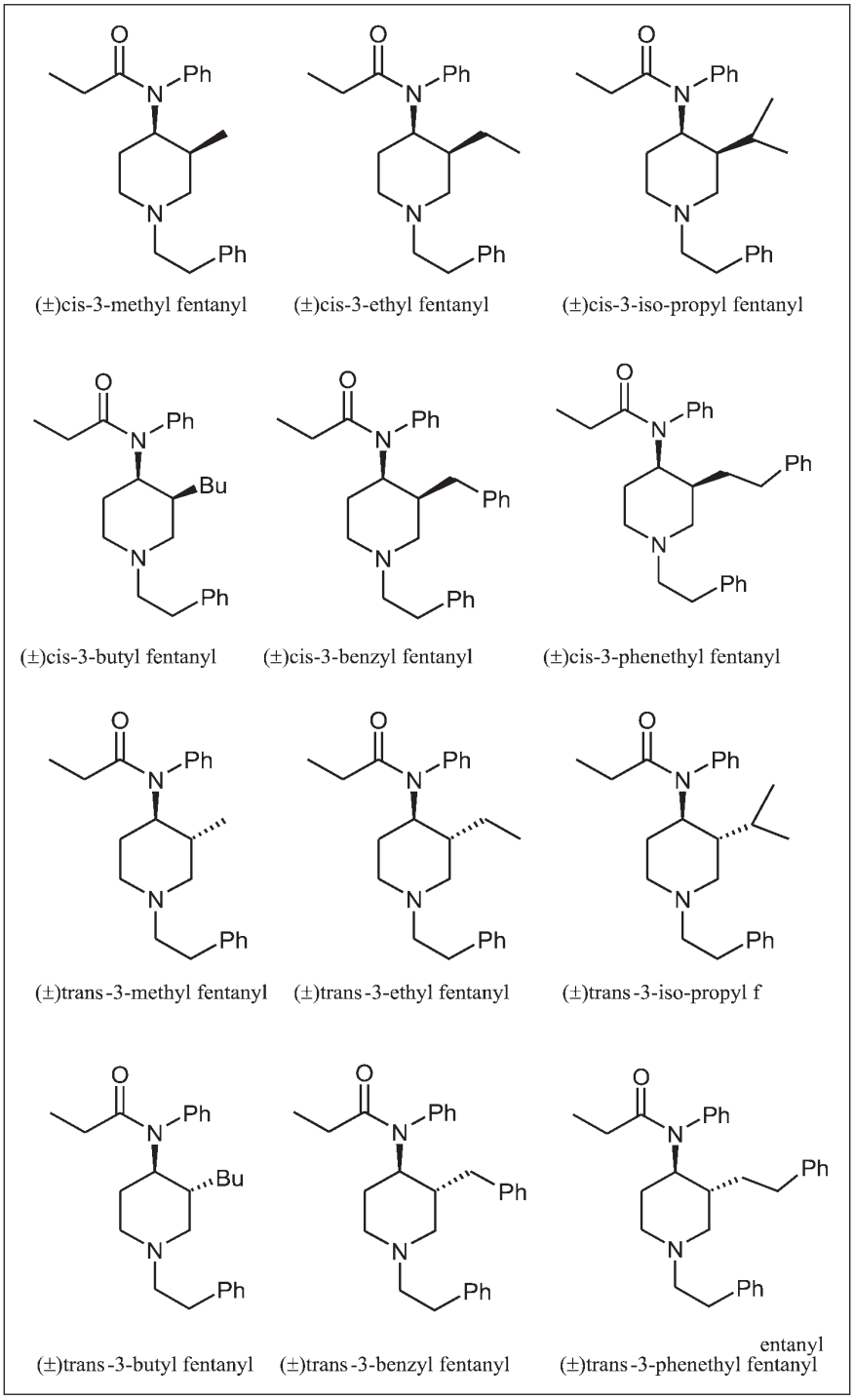

Figure 1. Fentanyl and its 3-alkyl analogues.

re 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 all the time, except during testing. Rats were individually removed from their housing for each temperature/nociception testing and returned immediately afterward. During measurements rats were restrained in plexiglas holders for about 15 seconds. This provided similar level of restraint and distress during antinociceptive and temperature measurements. Each compound was tested by using at least three doses. Experimental groups consisted of 6–8 rats. Each animal was used only once and was killed with intraperitoneal (i.p.) injection of sodium thiopental.

The antinociceptive activity was determined by "tail-immersion" test [19]. 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. In order to minimize tissue damage by repeated testing, the cut-off time was 10 sec. Pre-drug response latency was obtained 10-15 min before i.p. drug administration. Post-drug response latency was measured after i.p. administration of test compound. Response latency is expressed as a percent maximum possible effect (%MPE) and calculated according to the following formula: %MPE = (post-drug latency) - (pre-drug latency)/(cut-off time) - (pre-drug latency) x 100. Dose-response curves were analyzed using linear regression.

Fentanyl citrate, (±)*cis*- and (±)tra*ns*-3-methyl fentanyl oxalate, (±)*cis*- and (±)*trans*-3-ethyl fentanyl oxalate, (±)*cis*- and (±)*trans*-3-butyl fentanyl oxalate, (±)*cis*- and (±)*trans*-3-benzyl fentanyl oxalate and (±)*cis*-3-*iso*-propyl fentanyl, and (±)*cis*-3-phenethyl fentanyl (Faculty of Chemistry, University of Belgrade, Belgrade, Serbia) [20] were dissolved in saline and injected i.p. in a final volume of 2 ml/kg. All fentanyl analogues tested were examined as a racemic mixture. Naloxone hydrochloride (Sigma-Aldrich Chemical Co., St Louis, Mo., USA) was also dissolved in saline, and injected subcutaneously (s.c., 1 mg/kg) in the back 10 min before the i.p. injection of the test compound in the same volume. Doses of the drugs were calculated for the free base.

The ED_{50} (the dose that produces 50% of the effect) and 95% confidence limits were estimated from dose-response curve by using a standard statistical software [21]. Relative potency estimates were considered statistically significant when 95% confidence limits did not overlap 1.0. The duration of action was expressed as a time which is necessary for the tail withdrawal response to reduce to 50% MPE after i.p. injection of equi-effective doses (dose that produce 99% of the effect=ED_{99}) of tested compounds. To test whether saline injection in control rats has any effect on the tail immersion latency, the t-test for paired values was used. A P value of less than 0.05 was considered statistically significant.

RESULTS

All tested compounds showed time- and dose-dependent increase in antinociception (Fig. 2 and 3). The relative

potency was: (±)cis-3-methyl fentanyl (8) > (±)*trans*-3-methyl fentanyl (2) ≥ (±)*cis*-3-ethyl fentanyl (1.5) > fentanyl (1) ≥ (±)*trans*-3-ethyl fentanyl (0.9) > (±)*cis*-3-butyl fentanyl (0.064) ≥ (±)*trans*-3-butyl fentanyl (0.035) > (±)*cis*-3-benzyl fentanyl (0.008) ≥ (±)*trans*-3-benzyl fentanyl (0.0055). In summary, (±)*cis*-and (±)*trans*-3-methyl fentanyl and (±)*cis*-3-ethyl fentanyl are significantly (P<0.05) more potent, (±)*trans*-3-ethyl fentanyl is equipotent, while (±)*cis*- and (±)trans-3-butyl fentanyl, (±)*cis*- and (±)trans-3-benzyl fentanyl are significantly (P<0.05) less potent in comparison with fentanyl (Table 1; Fig. 3). (±)C*is*-3-*iso*-propyl fentanyl, and (±)*cis*-3-phenethyl fentanyl are inactive in doses up to 5 mg/kg (not shown).

Table 1. Summary of ED_{50} for antinociception and relative potencies of fentanyl and 3-substituted analogues of fentanyl in rats
Tabela 1. Pregled ED_{50} za antinocicepciju i relativne jačine fentanila i 3-supstituisanih analoga fentanila kod pacova

COMPOUND	ED_{50} (mg/kg)[1] (confidence limits)	Potency ratio	Time to peak[2] (min)	Duration of antinocic eption[3] (min)
fentanyl	0.0104 (0.006-0.018)	1	10	50*
(±)*cis*-3-methyl fentanyl	0.0013 (0.0012-0.0014)	8* (6.9-8.7)	15	90*
(±)*trans*- 3-methyl fentanyl	0.0053 (0.004-0.006)	1.97* (1.7-2.3)	10	40
(±)*cis*-3-ethyl fentanyl	0.0068 (0.0026-0.018)	1.5* (1.2-2.0)	15	60
(±)*trans*- 3-ethyl fentanyl	0.0116 (0.011-0.012)	0.90 (0.78-1.8)	10	40
(±)*cis*-3-butyl fentanyl	0.162 (0.082-0.320)	0.064* (0.05-0.08)	20	50
(±)*trans*-3-butyl fentanyl	0.348 (0.181-0.669)	0.035* (0.018-0.067)	20	50
(±)*cis*-3-benzyl fentanyl	1.31 (0.70-2.46)	0.008* (0.006-0.01)	30	50
(±)*trans*-3-benzyl fentanyl	1.91 (0.39-9.4)	0.005* (0.004-0.008)	40	50

[1]All ED_{50} values are expressed as free base weight.. [2]Time of maximum antinociception was meassured after i.p. injection of equi-effective doses (ED_{99}) of compound tested. [3]The duration of action was expressed as a time which is necessary for the tail withdrawal response to reduce to 50% MPE after i.p. injection of equi-effective doses (ED_{99}) of compounds tested. *P<0.05 in comparison with fentanyl.

Maximal analgesic responses were obtained 5-40 min after i.p. injection (ED99) of tested compound (Table 1, Fig. 2 and 3). The duration of action (ED99) was: (±)*cis*-3-methyl fentanyl (90 min) > (±)*trans*-3-methyl fentanyl (40 min) ≤ (±)*cis*-3-ethyl fentanyl (60 min) ≥ (±)*trans*-3-ethyl fentanyl (40 min) ≤ fentanyl (50 min) = (±)*cis*-3-butyl fentanyl (50 min) = (±)*trans*-3-butyl fentanyl (50 min) = (±)*cis*-3-benzyl fentanyl (50 min) = (±)*trans*-3-benzyl fentanyl (50 min). In summary, (±)*cis*-3-methyl fentanyl possesses significantly ($P<0.05$) longer duration of antinociception (after i.p. injection of ED99) in comparison with fen-

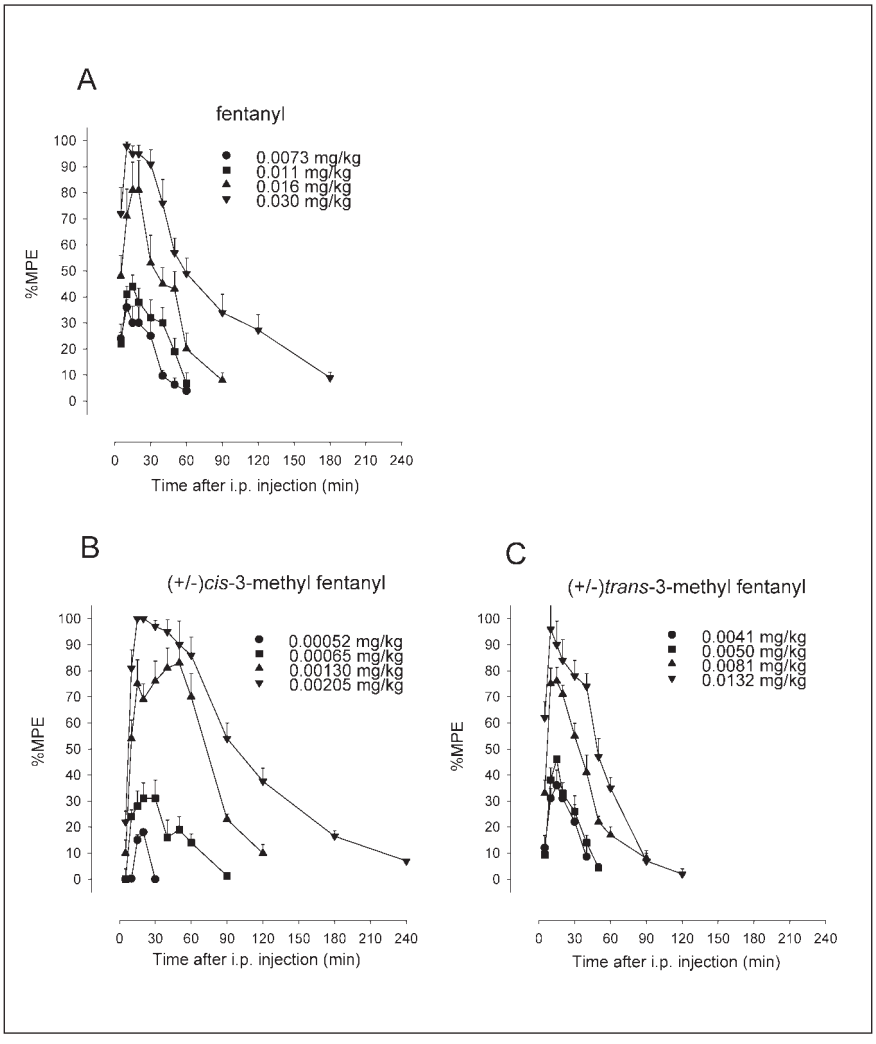

Figure 2. The time-effect curves on the tail immersion for different doses of fentanyl, (±)*cis*-3-methyl fentanyl and (±)*trans*-3-methyl fentanyl given intraperitoneally in rats. MPE = maximum possible effect. Each point represents the mean + S.E.M. of the antinociception in six to eight rats.

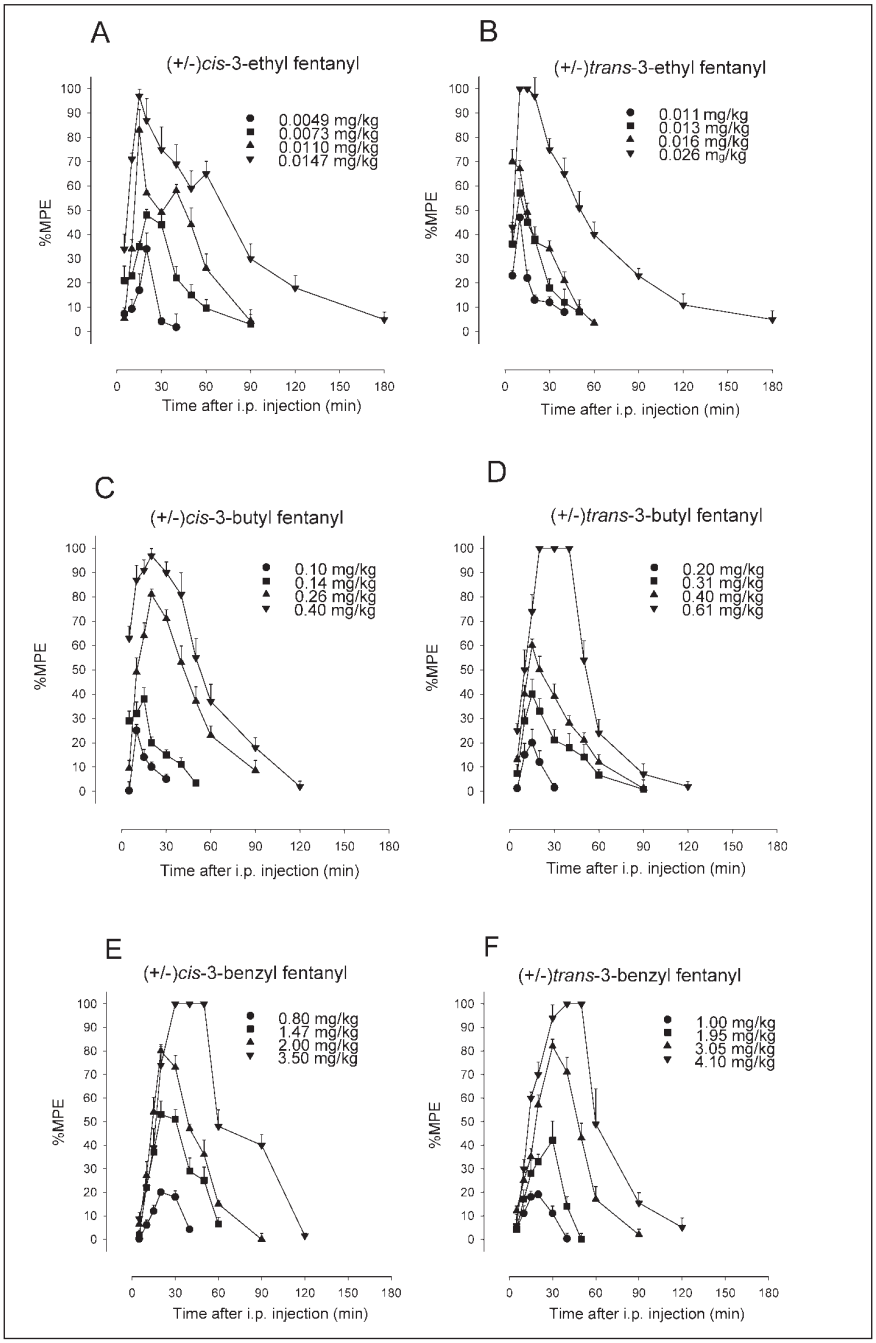

Figure 3. The time-effect curves on the tail immersion for different doses of (±)*cis*-3-ethyl fentanyl, (±)*trans*-3-ethyl fentanyl, (±)*cis*-3-butyl fentanyl, (±)*trans*-3-butyl fentanyl, (±)*cis*-3-benzyl fentanyl and (±)*trans*-3-benzyl fentanyl given intraperitoneally in rats. MPE = maximum possible effect. Each point represents the mean + S.E.M. of the antinociception in six to eight rats.

tanyl (Table 1, Fig. 2). The duration of action of the other 3-alkyl fentanyl analogues tested is similar to fentanyl (Table 1, Fig. 3).

DISCUSSION

A very large number of fentanyl analogues have been synthesized since 1963 [19]. The corresponding pharmacological data have been published and the structure-activity relationship established [2 - 9]. The synthesized 3-alkyl fentanyl analogues, all in the racemic form, were tested for antinociceptive activity and compared with the potency of fentanyl (Fig. 1). Those included two known compounds, *cis*-3-methyl fentanyl and *trans*-3-methyl fentanyl [22 - 24], as well as novel derivatives: *cis*-3-ethyl fentanyl, *trans*-3-ethyl fentanyl, *cis*-3-*iso*-propyl fentanyl, *cis*-3-butyl fentanyl, *trans*-3-butyl fentanyl, *cis*-3-benzyl fentanyl, *trans*-3-benzyl fentanyl and *cis*-3-phenethyl fentanyl (Fig. 1).

Based upon the results of the pharmacologic testing results of novel 3-alkyl fentanyl analogues and in agreement with previously published data [16 - 18], some tentative conclusions on the structure - activity relationship of 3-alkyl analogues of fentanyl were drawn:

- The presence of an alkyl group substituent in position 3 of the piperidine ring generally decreases or completely inhibits the analgesic activity compared to fentanyl. The exceptions are the (+)-*cis*-3-methyl analogue (≈ 19 x fentanyl) [17] and (±)-*cis*-3-ethyl fentanyl (≈ 1.5 x fentanyl) (Table 1) [19]. In the latter instance, presumably, one of the enantiomers was much more active than the other. Also, with the exception of racemic *cis*-3-methyl, and to a certain degree *cis*-3-ethyl fentanyl, the presence of 3-alkyl group, did not influence the duration of analgesic activity compared to fentanyl (Table 1).
- With increasing voluminosity of the alkyl group, the potency decreases rapidly. Thus, (±)-*cis*-3-propyl fentanyl, (±)-*cis*-3-butyl fentanyl, and (±)-*cis*-3-benzyl fentanyl, are 2, 16 and 126 times less potent than fentanyl, respectively. Derivatives which are even more bulky, (±)*cis*-3-*iso*-propyl fentanyl, and (±)*cis*-3-phenethyl fentanyl, are inactive in doses up to 5 mg/kg (not shown) [17]. Contrary to this, the duration of action of 3-alkyl fentanyl analogues, generally does not depend on the voluminosity of alkyl group (Table 1).
- The relative *cis*/*trans* stereochemistry is important since the *cis* isomers are 1.5–6 times more active than the *trans* isomers (Table 1) [17]. However, with the exception of 3-methyl fentanyl, and to a certain degree 3-ethyl fentanyl, the duration of action of equipotent analgesic doses of 3-alkyl fentanyl analogues is not significantly affected by relative stereochemistry (Table 1).
- The absolute stereochemistry appears to be critical, judging from the fact that (+)-*cis*-3-methyl fentanyl is about 100 times more active than the (–) enantiomer. Due to insufficient data on duration of

action of (+) and (-) enantiomers of 3-alkyl analogues, the influence of absolute stereochemistry on duration of action cannot be determined.

- At equianalgesic doses, more potent 3-alkyl analogues ((±)-cis-3-methyl fentanyl and (±)-cis-3-ethyl fentanyl) exerts longer duration of action in comparison to fentanyl and less potent 3-alkyl analogues. This finding might suggest that duration of action is more likely influenced by pharmacodynamic, than pharmacokinetic variables, as it has been already shown in the cases of (+)*cis*-3-methyl fentanyl [22 - 24], buprenorphine [25,26], *etc*.
- In accordance with the experiments done using fentanyl i.p. in rats (5) the side effects of 3-alkyl fentanyl analogues observed in this 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. Also, in preliminary testing, it has been revealed that the relative potencies that produce neurotoxic effects of fentanyl analgues substituted in position 3 of the piperidine ring are similar to the relative potencies used to induce analgesia [5].

In this study, time-response curves for antinociception were determined for each test compound in the presence of naloxone (not shown). Since all analogues were sensitive to naloxone antagonism, it was concluded that the antinociceptive effects are opioid-receptor-mediated.

It should be stressed, however, that the doses of compounds needed to produce maximal antinociception did not have any significant effect on motor the function, nor was any death observed during the 7 post-treatment hours.

CONCLUSION

In conclusion, the analgesic potency of 3-alkyl fentanyl analogues is influenced by the steric factor (voluminosity of the group at the position 3 of the piperidine ring and the *cis/trans* isomerism). Otherwise, with the exception of 3-methyl fentanyl, the duration of action of 3-alkyl fentanyl analogues is not significantly affected by the stereochemistry.

3-ALKIL ANALOZI FENTANILA: STUDIJA ODNOSA STRUKTURE I AKTIVNOSTI

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Kratak sadržaj

Uvod. Fentanil pripada grupi sintetskih opioidnih analgetika, 4-anilidopiperidina. Karakteriše ga visoka potentnost, brz početak i kratko trajanje dejstva. Do sada je sintetisan veliki broj analoga fentanila, kako u cilju određivanja odnosa strukture i farmakološke aktivnosti, tako i u cilju pronalaženja novog klinički korisnog analgetika.

Cilj rada. U studiji su ispitivana analgetska dejstva novosintetisanih 3-alkil analoga fentanila i poređena sa fentanilom.

Metod rada. Merenje analgetskog dejstva ispitivanih jedinjenja vršeno je uz pomoć testa potapanja repa pacova u toplu vodu.

Rezultati. Relativna jačina jedinjenja iznosila je: (±)*cis*-3-metil fentanil (8) > (±)*trans*-3-metil fentanil (2) ≥ (±)*cis*-3-etil fentanil (1,5) > fentanil (1) ≥ (±)*trans*-3-etil fentanil (0,9) > (±)*cis*-3-butil fentanil (0,064) ≥ (±)*trans*-3-butil fentanil (0,035) > (±)*cis*-3-benzil fentanil (0,008) ≥ (±)*trans*-3-benzil fentanil (0,0055). (±)Cis-3-izopropil fentanil i (±)cis-3-fenetil fentanil nisu ispoljili dejstvo u dozama do 5 mg/kg. Dužina dejstva (ED_{99}) iznosila je: (±)*cis*-3-metil fentanil (90 min) > (±)*trans*-3-metil fentanil (40 min) ≤ (±)*cis*-3-etil fentanil (60 min) ≥ (±)*trans*-3-etil fentanil (40 min) ≤ fentanil (50 min) = (±)*cis*-3-butil fentanil (50 min) = (±)*trans*-3-butil fentanil (50 min) = (±)*cis*-3-benzil fentanil (50 min) = (±)*trans*-3-benzil fentanil (50 min). Oznake > i < označavaju $P<0.05$.

Zaključak Zaključeno je da na analgetsku jačinu 3-alkil analoga fentanila utiče sterni faktor (voluminoznost grupe na položaju 3 piperidinskog prstena i *cis*/*trans* izomerizam). Inače, uz izuzetak 3-metil fentanila, stereohemija ne utiče značajno na dužinu dejstva 3-alkil analoga fentanila.

Ključne reči: fentanil, analozi, struktura, analgetska aktivnost

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