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Pharmacological studies of 3-(3-methyl-3-butenyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-8-hydroxy-2,6-methano-3-benzazocine.

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Pharmacological studies of 3-(3-methyl-3-butenyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-8-hydroxy-2,6-methano-3-benzazocine.*

Nobuo Nakamura

Abstract

3-(3-methyl-3-butenyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-8-hydroxy-2,6-methano-3-benzazocine (KF-1820) is a derivative of benzomorphan and is different from pentazocine only in the site of the double bond. KF-1820 showed potent analgesic activity in all tests performed. KF-1820 was 6 to 12 times and 30 to 40 times more potent than morphine and pentazocine, respectively, when administered subcutaneously. KF-1820 had little or no narcotic antagonist property. Physical dependence liability tests indicated that KF-1820 may be a little less, or as liable as, pentazocine to produce physical dependence. ID₅₀ values of KF-1820, pentazocine and morphine for depression of contractions of isolated guinea pig ileum to coaxial stimulation correlated well with their analgesic activities in the rodent. The dissociation equilibrium constant of KP-1820 confirmed the in vivo finding that KF-1820 had little or no narcotic antagonist property.

KEYWORDS: KF-1820, analgesic, non-narcotic antagonism, dependence, guinea pig ileum.

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**PHARMACOLOGICAL STUDIES OF 3-(3-METHYL-3-BUTENYL)-
1,2,3,4,5,6-HEXAHYDRO-6,11-DIMETHYL-8-HYDROXY-2,
6-METHANO-3-BENZAZOCINE**

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Abstract. 3-(3-methyl-3-butenyl)-1,2,3,4,5,6-hexahydro-6, 11-dimethyl-8-hydroxy-2,6-methano-3-benzazocine (KF-1820) is a derivative of benzomorphan and is different from pentazocine only in the site of the double bond. KF-1820 showed potent analgesic activity in all tests performed. KF-1820 was 6 to 12 times and 30 to 40 times more potent than morphine and pentazocine, respectively, when administered subcutaneously. KF-1820 had little or no narcotic antagonist property. Physical dependence liability tests indicated that KF-1820 may be a little less, or as liable as, pentazocine to produce physical dependence. ID₅₀ values of KF-1820, pentazocine and morphine for depression of contractions of isolated guinea pig ileum to coaxial stimulation correlated well with their analgesic activities in the rodent. The dissociation equilibrium constant of KF-1820 confirmed the *in vivo* finding that KF-1820 had little or no narcotic antagonist property.

Key words : KF-1820, analgesic, non-narcotic antagonism, dependence, guinea pig ileum.

In the search for alternative analgesics to morphine, interest attaches to a drug that has a potent analgesic activity but is devoid of physical dependence and psychotomimetic effects. 3-(3-methyl-3-butenyl)-1,2,3,4,5,6-hexahydro-6, 11-dimethyl-8-hydroxy-2,6-methano-3-benzazocine (KF-1820) is a derivative of benzomorphan synthesized for this purpose and is different from pentazocine only in the site of the double bond in the side chain.

In the preliminary experiments KF-1820 showed a potent inhibitory effect on thermally produced pain, which was not observed with pentazocine despite the structural similarity. The present study is an evaluation of the analgesic activity, narcotic antagonist property and physical dependence liability of KF-1820.

It has been shown (1-3) that the relative agonist potency in the guinea pig ileum is highly correlated with the analgesic potency in man and that the correlation between the relative antagonist potency in the guinea pig ileum and that in the morphine-dependent monkey is very close. Another aim of this study is therefore to compare the analgesic action and antagonist activity of KF-1820 obtained *in vivo* with those in the isolated ileum of guinea pig.

MATERIALS AND METHODS

The experiments were conducted using male albino mice (dd strain), male albino rats (Wistar strain), male guinea pigs and male rabbits.

Acute Toxicity.

Each drug was administered subcutaneously or orally to a group of 10 mice (20 ± 1 g) or 10 rats (90 ± 10 g). The LD₅₀ value was estimated 7 days after administration.

Analgesic Activity.

Acetic acid-induced writhing test. A modification of the procedure described by Koster *et al.* (4) was used. A group of 10 mice (20 ± 1 g) was injected intraperitoneally with a 0.7% acetic acid (0.1 ml/10g) concurrently with subcutaneous injection or oral administration of the test drug (Only morphine was given orally 15 min before the administration of acetic acid). The number of the writhings for each mouse was counted for 10 min beginning 10 min after the acetic acid injection. The test drug was considered to be effective if it depressed the number of writhings to less than 50% of the mean number obtained in control animals.

Intra-arterial bradykinin injection test. The experiments were carried out using rats (210-260 g) according to the procedure described by Deffnu *et al.* (5) and Abe *et al.* (6). A polyethylene cannula of 0.5 mm in diameter was introduced in a retrograde manner into the right carotid. The end of the cannula was subcutaneously led toward the scapular, put outside the body and connected with a short-cut intravenous needle with a rubber stopper. Bradykinin (0.4-0.6 μ g in 0.2 ml) was rapidly injected (within 1 sec or less) into the right carotid through the cannula at intervals of 15 min. An intra-arterial injection of bradykinin immediately produced such abnormal behaviors in rats as flexion of the right forelimb, rotatory movement of the head and hyperactivity. The test drug was considered to be effective if it inhibited all or two of the three bradykinin-induced behaviors.

Tail flick test. Analgesic activities in mice (20 ± 1 g) and rats (90 ± 10 g) were determined by the radiant heat method of D'Amour and Smith (7). Only mice and rats withdrawing their tail from the heat within 7 sec were used. The response time was measured 15, 30, 60 and 90 min after administration. If the response time was more than 15 sec, the test drug was considered to be effective.

Tail pinch test. Mice (20 ± 1 g) were pinched at the root of the tail with an arterial clamp according to the method of Takagi (8). Only mice biting their tail within 2 sec were used. The test drug was considered to be effective if the response time was more than 6 sec.

Tooth pulp stimulation test. Rabbits (about 2.5 kg) were anesthetized with pentobarbital sodium (30 mg/kg i.v.). To stimulate the tooth pulp electrically, a small opening was drilled in the dentine of the lower incisor and bipolar stainless steel electrodes (0.25 mm in diameter) were inserted into the hole and fixed with dental cement. Cortical and subcortical stainless steel electrodes were implanted to record EEGs. The sites of subcortical electrodes (dorsal hippocampus, nucleus amygdalae and mesencephalic reticular formation) were determined according to the atlas of Sawyer *et al.* (9). The experiments were performed using unanesthetized rabbits after more than a week of recovery period. EEG arousal responses were induced by electrical stimulation (60 Hz, 0.1 msec, period of stimulation 5 sec) to the tooth pulp and to the mesencephalic reticular formation (100 Hz, 1 msec, 7 sec). EEGs were recorded using a Nihon Kohden Electroencephalograph and the stimulating current was provided by a Nihon Kohden Stimulator (MSE-3) in connection with an isolating unit.

Narcotic Antagonist Activity.

Antagonism of morphine analgesia. Analgesia was measured 15, 30, 60 and 90 min after the subcutaneous injection of a combination of morphine and KF-1820 or pentazocine by the rat tail flick method described already.

Antagonism of morphine-induced respiratory depression. The ability to reverse morphine-induced respiratory depression was used as an assessment of narcotic antagonist activity. Rabbits (2 to 2.5 kg) were anesthetized with urethane (1 g/kg i.p.). The respiratory rate and pressure of expired air were recorded on a polygraph (Nihon Kohden RM-85) through a force transducer. KF-1820 or pentazocine was injected intravenously 3 min after the intravenous injection of morphine 8 mg/kg.

Action of Levallorphan

The experiment was performed using the rat tail flick method described already. The antagonist action of levallorphan on KF-1820- or morphine -analgesia was assessed after concurrent injection. All drugs were administered subcutaneously.

Physical Dependence Liability

Primary dependence test. Rats (120 ± 10 g) were given a drug s.c. twice daily at 08:00 and 16:00 for more than 7 weeks according to the method of Hosoya *et al.* (10). The dose was increased weekly until the maximum tolerated dose or the maintenance dose was reached, as shown in Table 1. A withdrawal sign precipitated by cessation of the drug after 4 consecutive weeks of administration was assessed. Body weight loss was used as the withdrawal sign.

TABLE 1. DOSE SCHEDULE OF CHRONIC ADMINISTRATION OF TEST DRUGS IN THE PRIMARY DEPENDENCE TEST

Drugs	1 week	2 week	3-8 week
KF-1820			
(L)	0.5	1	2
(M)	2	5	10
(H)	5	10	20
Pentazocine			
(L)	2	5	10
(H)	5	10	20
Morphine			
(L)	5	10	20
(H)	10	20	40

mg/kg s.c.

(L): Low doses; (M): Medium doses; (H): High doses

Two-dose substitution test. Rats, weighing 140-160 g at the beginning of the experiment, were administered with morphine twice daily (at 08:00 and 16:00) for 8 weeks according to the dose schedule shown in Table 2. Morphine-dependent rats, receiving morphine s.c. at a maintenance dose of 80 mg/kg after 4 weeks of increasing doses, were given KF-1820 or pentazocine s.c. morning and evening on one day in place of morphine according to the method of Martin (11) and Nakamura *et al.* (12). The body weight was measured 8 and 16 h after the second administration of KF-1820 or pentazocine.

TABLE 2. DOSE SCHEDULE OF CHRONIC ADMINISTRATION OF MORPHINE IN THE TWO DOSES SUPPRESSION TEST

	1 week	2 week	3 week	4-8 week
Morphine	10	20	40	80

mg/kg s.c.

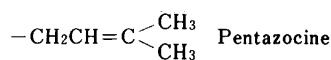
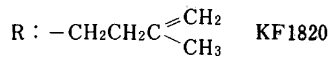
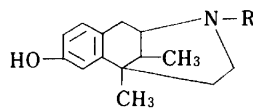
Effect on Contractions of Isolated Guinea Pig Ileum to Coaxial Stimulation

All experiments were performed on isolated guinea pig ileum. The detail procedure differed slightly from that described by Kosterlitz and Watt (13). The terminal portion of the ileum was used while the 10 cm nearest to the ileo-cecal junction was discarded. The depressant action of a drug was tested on the contraction of the longitudinal muscle induced by coaxial electrical stimulation. The bath fluid, Tyrode's solution (30 ml) to which hexamethonium bromide (69 μM) and tripeleennamide hydrochloride (0.125 μM) were added, was bubbled with 95% oxygen and 5% carbon dioxide and its temperature was maintained at 37°C. The stimuli were supramaximal rectangular pulses of 0.5 msec duration at a frequency of five per minute. Values of ID_{50} , agonist activity, were determined from log dose-response curves according to the formula reported by Kosterlitz *et al.* (13). Antagonist activity was measured by determining the dissociation constant K_e which is obtained from $K_e = a / (\text{DR} - 1)$ where 'a' is the concentration of the drug with antagonist activity and 'DR' is the ratio of concentrations of the agonist required to give the same depression of twitch in the presence and absence of the antagonist. The effective antagonist potency (Pa) which took into account the agonist activity of partial agonist was expressed thus by $\text{Pa} = \text{ID}_{50} / K_e$.

Drugs

Drugs used were 3-(3-methyl-3-butenyl)-1,2,3,4,5,6-hexahydro-6, 11-dimethyl-8-hydroxy-2,6-methano-3-benzazocine (KF-1820, Kyowa Hakko Kogyo Co., Ltd.), pentazocine (Yamanouchi Pharmaceutical Co., Ltd.), morphine hydrochloride (Takeda Chemical Ind., Co., Ltd.), levallorphan (Takeda Chemical Ind., Co., Ltd.) and synthetic bradykinin (Institute for Protein Research, Osaka University).

Chemical structures

*Statistical Analysis*

LD_{50} values for lethality, ED_{50} values for analgesia and 95% limits were calculated from the rates of 4 to 6 doses according to the method of Litchfield and Wilcoxon (14).

RESULTS

Acute Toxicity

The LD₅₀ values of KF-1820 and pentazocine are shown in Table 3. In the mouse, KF-1820 was about 1.5 times as toxic as pentazocine both by subcutaneous injection and by oral administration. In the rat, it was about three times as toxic as pentazocine by subcutaneous injection.

TABLE 3. ACUTE LETHALITY (LD₅₀) IN MOUSE AND RAT

Drug.	LD ₅₀ mg/kg	
	Mouse	Rat
KF-1820		
s.c.	80 (64-101)	61 (39-95)
p.o.	205 (166-253)	>300
Pentazocine		
s.c.	140 (118-166)	165 (134-204)
p.o.	305 (259-359)	>400

() : confidence limit

Analgesic Activity

Acetic acid-induced writhing test. The ED₅₀ values for inhibition of acetic acid-induced writhing are shown in Table 4 for the three drugs tested. KF-1820 inhibited the writhings more strongly than pentazocine and morphine, since, in the case of subcutaneous injection, it was about 6 times more active than morphine and about 40 times more active than pentazocine. It was about 1/3 times more active than morphine and about 6 times more active than pentazocine by oral administration.

Intra-arterial bradykinin infection test. Time-courses of inhibitory actions of KF-1820, pentazocine and morphine on abnormal behaviors induced by intra-arterial injection of bradykinin are illustrated in Fig. 1. The duration of inhibitory action after subcutaneous injection was comparable among the three drugs tested. KF-1820 administered subcutaneously was about 12 times as potent as morphine and about 27 times as potent as pentazocine. In the case of oral administration, KF-1820 was about twice as active as morphine and more than three times as active as pentazocine (Table 4).

Tail flick test. Table 4 shows the ED₅₀ values of the drugs in this test. When injected subcutaneously, KF-1820 was 6 to 7 times more potent than morphine in the mouse as well as in the rat. Pentazocine was inactive at a dose as high as 40 mg/kg.

TABLE 4. ANALGESIC ACTIVITY (ED₅₀) OF KF-1820, PENTAZOCINE AND MORPHINE

Drugs	Test ED ₅₀ (mg/kg)				
	Acetic acid writhing Mouse	Bradykinin test Rat	Tail-flick test		Tail-pinch test Mouse
			Mouse	Rat	
KF-1820					
s.c.	0.09 (0.07-0.11)	0.38 (0.27-0.54)	0.76 (0.64-0.90)	0.48 (0.37-0.63)	0.51 (0.43-0.60)
p.o.	14.3 (10.6-19.3)	35 (25 - 49)	—	—	29.3 (22.9-37.5)
Pentazocine					
s.c.	3.45 (2.52-4.73)	10.5 (7.2-15.2)	>40	>40	>40
p.o.	82 (62 - 109)	>100	—	—	>40
Morphine					
s.c.	0.56 (0.41-0.77)	4.8 (3.9-5.9)	5.2 (4.5-6.0)	3.2 (2.4-4.1)	3.5 (2.7-4.6)
p.o.	5.1 (3.9-6.6)	85 (58-125)	—	—	35.5 (25.4-49.7)

Numbers in parentheses indicate confidence limit.

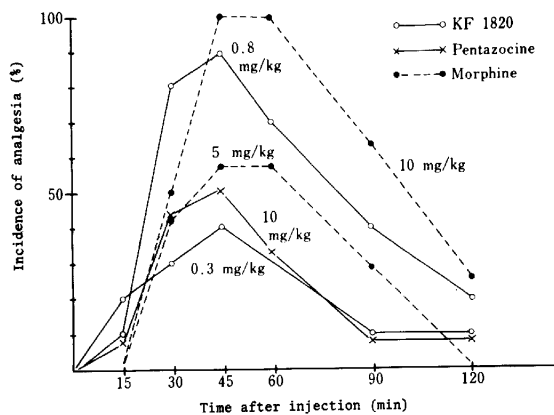


Fig. 1. Time course for inhibition of bradykinin-induced behavior following the s.c. injection of KF-1820, pentazocine and morphine.

Tail pinch test. KF-1820 was more potent as an antinociceptive agent than pentazocine and morphine in the tail pinch test. The data in Table 4 show that KF-1820 administered subcutaneously was about 7 times more potent than morphine and that both drugs were comparable in activity when given orally.

Tooth pulp stimulation test. The intravenous injection of KF-1820 produced a slow wave pattern on EEG at doses of more than 0.01 mg/kg. The dose of 0.1 mg/kg caused EEG changes consisting of an increase in synchronization and amplitude for about 60 min immediately after injection in all channels. Such behavior as biting, licking and head twitch and an arousal pattern on EEG were observed on electrical stimulation of 5 to 7 V to the tooth pulp. The threshold of EEG arousal response was little affected by the intravenous injection of KF-1820 (0.01 mg/kg). KF-1820 (0.1 mg/kg) elevated the threshold by about 150% (Figs 2 and 3). The intravenous injection of pentazocine (5 mg/kg) or morphine (2 mg/kg) caused about an 100% elevation in threshold. The intravenous injection of KF-1820 (0.1 mg/kg), pentazocine (5 mg/kg) or morphine (2 mg/kg) elevated the threshold of EEG arousal response to the stimulation of mesencephalic reticular formation by only about 30% (Fig. 3).

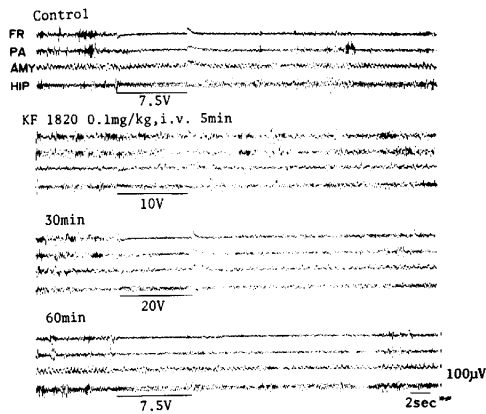


Fig. 2. Effect of KF-1820 on EEG arousal response produced by high rate stimulation of tooth pulp.

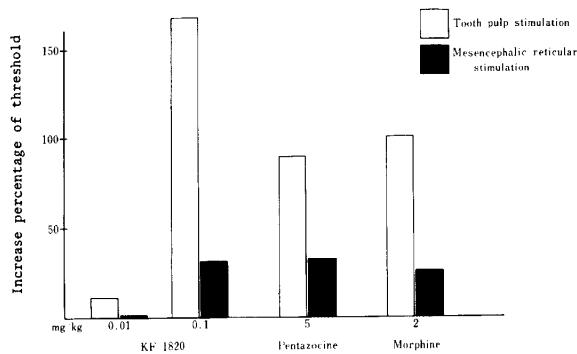


Fig. 3. Effects of KF-1820, pentazocine and morphine on EEG arousal response to tooth pulp and mesencephalic reticular stimulation in the rabbit.

Narcotic Antagonist Activity

Antagonism of morphine analgesia. When morphine at a dose of 7 mg/kg and pentazocine at various doses were injected concurrently by the subcutaneous route, morphine analgesia was antagonized by pentazocine in a dose-related way. On the other hand, KF-1820 potentiated morphine analgesia markedly (Table 5). Figure 4 illustrates the time-course of analgesia after a subcutaneous injection of the combination of morphine (2.5 mg/kg) and KF-1820 (0.25mg/kg). The combination showed more potent and longer lasting analgesia than either drug used alone.

TABLE 5. NARCOTIC ANTAGONIST ACTIVITY OF KF-1820 AND PENTAZOCINE. ANALGESIC ACTIONS OF THE COMBINATION OF MORPHINE WITH KF-1820 OR PENTAZOCINE. (TAIL-FLICK TEST: RAT)

	Control	KF-1820 (mg/kg s.c.)			Pentazocine (mg/kg s.c.)			
		0.25	0.5	0.7	5	10	25	70
Morphine	0	2/10	5/10	5/5	0/5	0/5	0/5	1/5
(mg/kg s.c.)	2.5	4/10	5/5	5/5	—	—	—	—
	7	10/10	—	5/5	5/5	4/5	2/5	2/5

Animals showing analgesia/Total animals

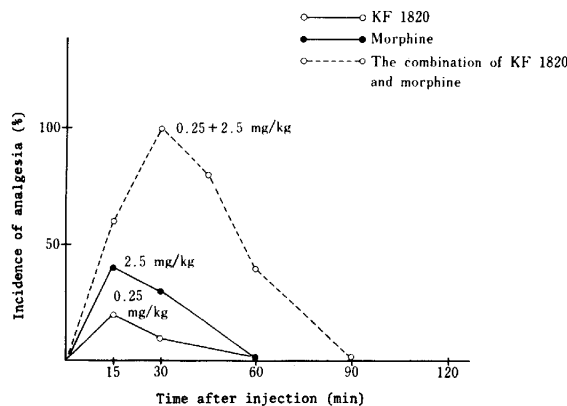


Fig. 4. Time course of analgesic actions of KF-1820, morphine or the combination of KF-1820 and morphine (Tail-flick Test:Rat).

Antagonism of morphine-induced depression in respiration. The intravenous injection of KF-1820 had little influence on the respiratory rate of rabbits at a dose of 0.01 mg/kg but depressed it slightly at a dose of 0.03 mg/kg. Long lasting depression of the respiratory rate was induced by KF-1820 (0.3 mg/kg). Intravenous pentazocine at doses ranging from 0.3 to 1 mg/kg depressed the respiratory rate. The intravenous injection of 8 mg/kg of morphine decreased the respiratory rate

but increased its amplitude. KF-1820 at dose levels ranging from 0.01 to 0.3 mg/kg did not antagonize but potentiated the depressive action of morphine (Fig. 5). Pentazocine showed a tendency toward antagonism against morphine at a dose of 0.3 mg/kg and showed remarkable antagonism at a dose of 1 mg/kg (Fig. 6).

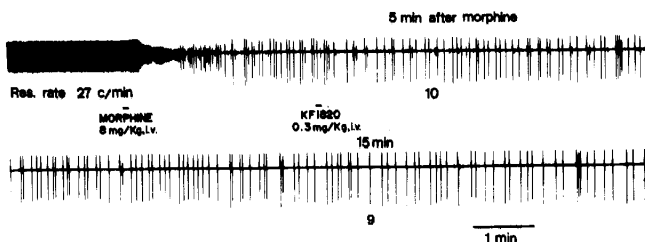


Fig. 5. Effect of KF-1820 on morphine-induced respiratory depression in rabbit.
Res. rate: Respiratory rate.

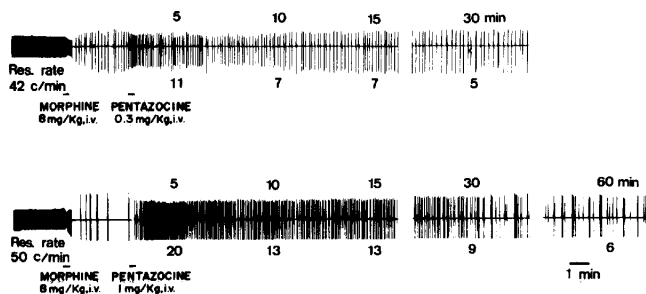


Fig. 6. Effect of pentazocine on morphine-induced respiratory depression in rabbit.
Res. rate: Respiratory rate.

Action of Levallorphan

Subcutaneous levallorphan when combined with KF-1820 at a dose of 1mg/kg or with morphine at a dose of 7 mg/kg, either dose level producing analgesic action to all rats tested, produced a dose-related antagonism against analgesia caused by either drug. The potency of antagonism was comparable between KF-1820 and morphine (Table 6).

Physical Dependence Liability

Primary dependence test. Rats chronically receiving KF-1820, pentazocine or morphine showed a decrease in body weight gain as the dose level increased (Fig. 7). Whenever morphine was withdrawn for 24 h every week after 4 consecutive weeks' administration, a remarkable loss of body weight by a little less than 10% of the whole body weight occurred abruptly. During the withdrawal period, the rats displayed sedation, diarrhea and loss of appetite. On the contrary, the loss of

body weight was not observed in rats chronically treated with KF-1820 or pentazocine on withdrawal. Rats treated with the highest dose of KF-1820 gained weight a little on withdrawal. The changes in body weight evoked by withdrawal, including the abrupt loss in morphine-treated rats and the slight increase in rats given the highest dose of KF-1820, disappear when either drug was administered again and the body weight recovered to normal.

TABLE 6. LEVALLORPHAN ANTAGONISMS OF KF-1820- OR MORPHINE-INDUCED ANALGESIA

	Control	Levallorphan mg/kg s.c.		
		0.25	0.5	2
KF-1820 1 mg/kg s.c.	5/5	4/5	2/5	0/5
Morphine 7 mg/kg s.c.	5/5	4/5	1/5	0/5

Animals showing analgesia / Total animals

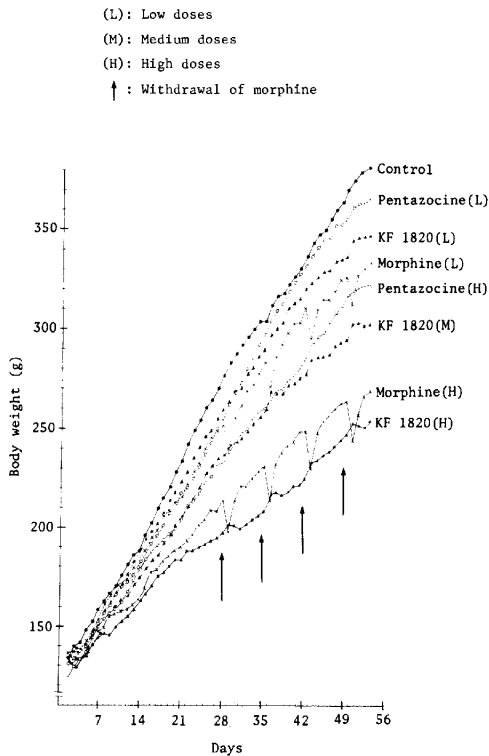


Fig. 7. Body weights during chronic administration of an increasing dose of KF-1820, pentazocine or morphine and withdrawal.

Two-dose substitution test. Morphine-dependent rats lost weight gradually during 40 h of withdrawal (Fig. 8). The subcutaneous injection of KF-1820 or pentazocine inhibited the loss of body weight occurring on withdrawal of morphine. In view of the inhibitory effect, KF-1820 at a dose of 1 mg/kg (A) was equivalent to pentazocine at a dose of 20 mg/kg (B) and KF-1820 at a dose of 10 mg/kg was equivalent to pentazocine at a dose of 50 mg/kg. The inhibitory effect induced by 10 mg/kg of KF-1820 or 50 mg/kg of pentazocine was marked. The animals that were continuously given the maintenance dose of morphine (80 mg/kg) did not lose but gained body weight (C).

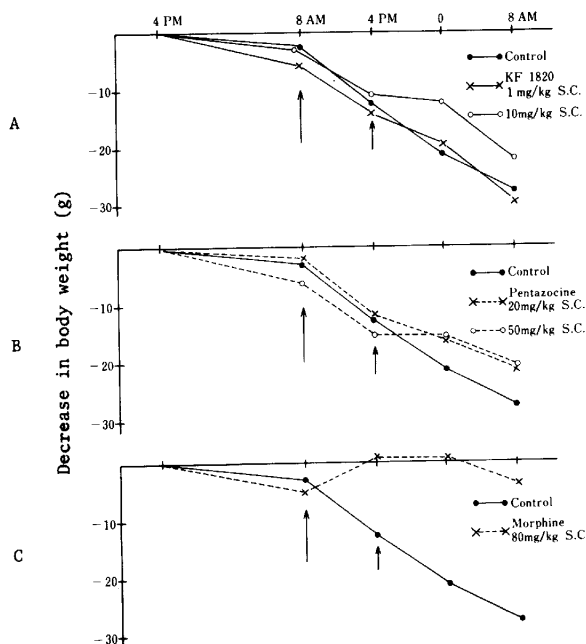


Fig. 8. The two doses suppression test in rats. Effects of cross-administration between morphine and test drugs. Test drugs were injected at the point indicated by the arrow.

Effect on Contractions of the Isolated Guinea Pig Ileum to Coaxial Stimulation

KF-1820, pentazocine and morphine inhibited the contractions of isolated guinea pig ileum evoked by coaxial electrical stimulation. As the ID_{50} values in Table 7 show, KF-1820 inhibited contractions about 6 times more potency than morphine and about 30 times more potency than pentazocine. Fig. 9 illustrates the effects of 10 and 30 nM of morphine, 3 nM of KF-1820 and 100 nM of pentazocine on contractions of the isolated ileum to coaxial stimulation. The inhibitory effect of morphine on the contraction was hardly affected by pre-administration of KF-1820, while it was fairly antagonized by pre-administration

of pentazocine (Fig. 10). The dissociation equilibrium constant K_e of KF-1820 was 10.8 nM and that of pentazocine was 3.6 nM. The effective antagonist potency (P_a) of KF 1820 was, however, much less than that of pentazocine because of its high agonistic activity (Table 7).

TABLE 7. KINETIC PARAMETERS OF KF-1820 AND PENTAZOCINE.

	ID_{50} (nM)	K_e (nM)	P_a
KF-1820	2.7± 0.5	10.8	0.25
Pentazocine	74.3± 26.4	3.6	20.6
Morphine	17.7± 3.0	—	—

Means and S.E. of means of agonist activity (ID_{50}) obtained from six observations.

K_e : Dissociation equilibrium constant; P_a : Effective antagonist potency

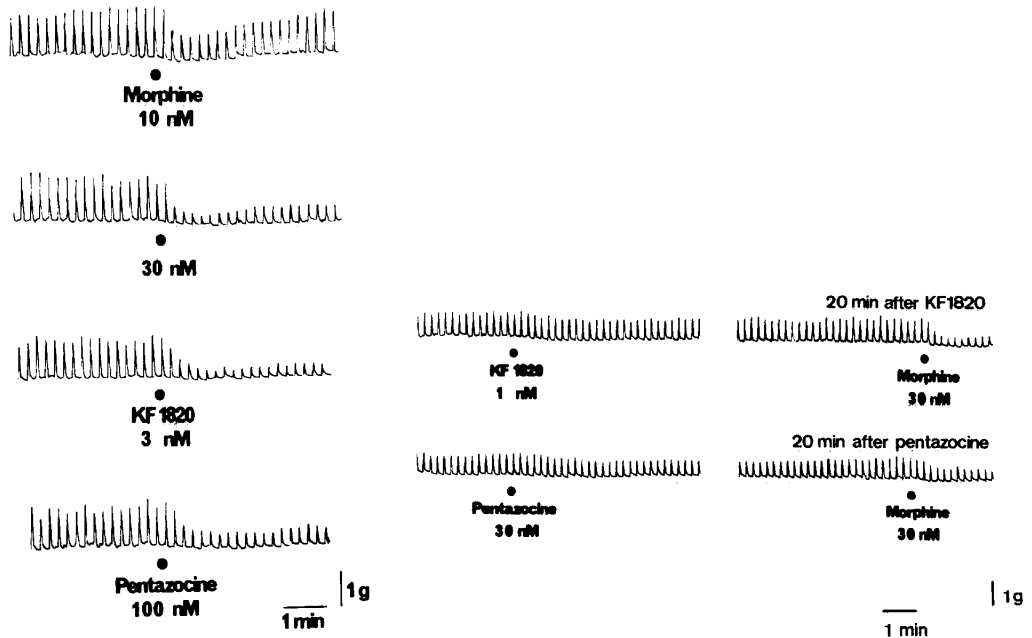


Fig. 9. (left) Depression of contractile responses of guinea pig ileum to coaxial stimulation by KF-1820, pentazocine or morphine.

Fig. 10. (right) Measurement of the antagonist activity of KF-1820 by determination of its equilibrium constant.

TABLE 8. SAFETY MARGIN OF KF-1820 AND PENTAZOCINE

Animals	KF-1820		Pentazocine	
	s.c.	p.o.	s.c.	p.o.
Mouse	888	14	41	4
Rat	161		16	

R = LD₅₀/ED₅₀, ED₅₀: Acetic acid writhing test, Bnadykinin test.

DISCUSSION

The continuous and extensive search for non-narcotic analgesics led to the finding that KF-1820 possessed a strong analgesic action. KF-1820 differs structurally from pentazocine only in the site of the double bond at the side chain. KF-1820 was found to be effective in all analgesic tests performed. When administered subcutaneously, it was 6 to 12 times more potent than morphine. In the chemical stimulation analgesic tests suitable for the evaluation of the analgesic activity of pentazocine, KF-1820 was 30 to 40 times more potent than pentazocine when administered subcutaneously. It has been reported (6, 15) that there is a close correlation between the analgesic activity obtained in chemical stimulation analgesic tests and the analgesic potency in man. It is reasonable, therefore, to predict a strong analgesic activity of KF-1820 in man.

In the rodent, the analgesic activity of subcutaneous or oral KF-1820 is so potent as to make its safety margin (LD₅₀/ED₅₀ for analgesia) considerably wider than that of pentazocine (Table 8), although KF-1820 is 1.5 to 3 times more toxic. This result suggests that the safety of KF-1820 may be higher than that of pentazocine in clinical usage.

It has been demonstrated (15, 16) that the analgesic activities of narcotic antagonists such as pentazocine, nalorphine and cyclazocine are not assessed by conventional tests (tail flick test and tail pinch test). KF-1820 is clearly different from pentazocine in respect of the result that it is active in these tests. Moreover, KF-1820 antagonized neither analgesia by morphine in the rat nor respiratory inhibition induced by morphine in the rabbit. These facts indicate that KF-1820 has little or no narcotic antagonist property. It has been shown that new synthetic analgesics including oxilorphan (17), buprenorphine (18), 20681-S, 20682-S (19) and 1-cyclohexyl-4-[2-(3-hydroxyphenyl)-1-phenylethyl] piperazine (20) are narcotic antagonists. Therefore, these drugs might be different in property from KF-1820.

Kosterlitz *et al.* (21) reported that the antagonist potency of naloxone, a pure antagonist, is lower against drugs with dual agonist and antagonist effects such as nalorphine and levallorphan than against morphine. This finding gives a sufficient explanation of the result of the experiments reported in this paper that the analgesic activities of KF-1820 and morphine were antagonized by levallorphan

to the same extent because KF-1820 was not considered a narcotic antagonist. However, the benzomorphans (Mr1268, Mr1353) without antagonist component and the endogenous peptide enkephalin which is an agonist without antagonist activity have been reported to require more naloxone for their antagonism (22, 23). Accordingly, it would appear that benzomorphan derivatives do not always act upon the same receptor. This can probably be explained by the proposal (24) that there are three types of opiate receptor (μ -receptor, κ -receptor and δ -receptor).

Akera and Brody (25) reported that the loss of body weight on withdrawal of the drug was the best index of addiction in rats. In the primary dependence test performed, considerable loss of body weight was seen in the morphine-treated group after withdrawal of the drug. Unlike the morphine-treated group, rats chronically treated with KF-1820 or pentazocine did not show any decrease in body weight after abrupt withdrawal. From these results, it is suggested that the physical dependence liabilities of KF-1820 and pentazocine are quite different in potency from that of morphine.

Rats chronically receiving the highest dose of KF-1820 gained body weight on discontinuation. This may be because during withdrawal the rats were relieved from the toxic effects of doses as high as 20 mg/kg s.c.

In the two-dose substitution test, the subcutaneous injection of KF-1820 at a dose of 10 mg/kg or pentazocine at a dose of 50 mg/kg prevented loss of body weight after withdrawal of morphine in morphine-dependent rats, indicating that KF-1820 and pentazocine can substitute for morphine. Our results with pentazocine are in accord with those of Nakamura *et al.* (12). Taking into account the analgesic activity of KF-1820 which is 30 to 40 times more potent than that of pentazocine, it is concluded that KF-1820 may be a little less liable than, or as liable as, pentazocine to produce physical dependence.

It is known (3) that morphine inhibits impulse transmission at certain junctions of the autonomic nervous system. These sites are not characteristic of species, organ, or tissue. The peristaltic reflex elicited by distension of the lumen of the isolated ileum is inhibited by morphine in the guinea pig but is unresponsive to morphine in the rabbit (26). A morphine-sensitive adrenergic junction has been found in the nictitating membrane of the cat and the vas deferens of the mouse, but not in the rat, guinea pig, rabbit or hamster (27). Kosterlitz *et al.* (3) have demonstrated that the potency of narcotic analgesics to depress the contractions of isolated guinea pig ileum and mouse vas deferens evoked by coaxial electrical stimulation as *in vitro* models correlated highly with their analgesic potency. Their antagonist potency was compared to the potency to cause withdrawal symptoms in the morphine-dependent monkey. These preparations have, therefore, been considered as simple and suitable models for the prediction of the agonist and antagonist properties of narcotic analgesics.

It has been reported (28) that there is good agreement between the agonist

potency of a narcotic analgesic estimated with guinea pig ileum and with mouse vas deferens, but the antagonist potency in the guinea pig is more closely correlated to the values obtained in the morphine-dependent monkey than to those for the vas deferens. Hughes (29) has concluded that as an assay model, the guinea pig ileum is more robust and consistent in its response than the mouse vas deferens.

The research using the *in vitro* preparations described above led to the discovery of endogenous opioid peptide (30). Recent studies (31,32) have demonstrated that Kyotorphin, a dipeptide isolated from the bovine brain, possesses a potent analgesic action in the mouse tail-pinch test but only slightly depresses contractions of the longitudinal muscle of the ileum evoked by coaxial electric stimulation. The lack of uniformity in the distribution of dihydromorphine and enkephalin receptors among longitudinal muscle of various parts of the ileum indicates the plurality of opiate receptors (33).

The potency to reduce stereospecific naloxone binding in brain homogenate has been demonstrated to agree with the dissociation equilibrium constant (K_e) obtained in guinea pig ileum (3, 34). Nevertheless, it remains to be elucidated whether the *in vitro* finding in the peripheral tissue, *i.e.* ileum and the central analgesic actions of opioids can be explained by the same mechanism. In the present experiment, ID_{50} values of KF-1820, pentazocine and morphine for the depression of contractions of the isolated guinea pig ileum to coaxial stimulation correlated well with their analgesic activities in the rodent. The dissociation equilibrium constant of KF-1820 confirmed the *in vivo* finding that KF-1820 had little or no narcotic antagonist property. It can be concluded that so far as opioid analgesics such as morphine, pethidine and pentazocine are concerned, the results obtained in the guinea pig ileum are highly predictive of their central analgesic and narcotic antagonist activities.

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REFERENCES

1. Gyang, E.A. and Kosterlitz, H.W.: Agonist and antagonist actions of morphine-like drugs on the guinea-pig isolated ileum. *Br. J. Pharmacol. Chemother.* **27**, 514-527, 1966.
2. Fennessy, M.R., Heimans, R.L.H. and Rand, M.J.: Comparison of effect of morphine-like analgesics on transmurally stimulated guinea-pig ileum. *Br. J. Pharmacol.* **37**, 436-449, 1966.
3. Kosterlitz, H.W. and Waterfield, A.A.: In vitro models in the study of structure-activity relationships of narcotic analgesics. *Annu. Rev. Pharmacol.* **15**, 29-47, 1975.
4. Koster, R., Anderson, M. and De Beer, E.J.: Acetic acid for analgesic screening. *Fed. Proc.* **18**, 412-413, 1959.
5. Deffenu, C., Pegrassi, L. and Lumachi, B.: The use of bradykinin-induced effects in rats as an assay for analgesic drugs. *J. Pharm. Pharmacol.* **18**, 135, 1966.

6. Abe, T., Kaneko, T. and Takagi, H.: Studies on the assessment of analgesic actions of narcotic antagonists with special reference to nociception induced by retrograde injection of bradykinin into the carotid artery of rat. *Folia Pharmacol. Jpn.* **67**, 9-14, 1971.
7. D'Amour, F.E. and Smith, D.L.: A method for determining loss of pain sensation. *J. Pharmacol. Exp. Ther.* **72**, 74-79, 1941.
8. Takagi, H., Inukai, T. and Nakama, M.: A modification of Haffner's method for testing analgesics. *Jpn. J. Pharmacol.* **16**, 287-295, 1966.
9. Sawyer, C.H., Everett, J.W. and Green J.D.: The rabbit diencephalon in stereotaxic coordinates. *J. Comp. Neurol.* **110**, 801-820, 1954.
10. Hosoya, E. and Otobe, S.: Study of drug addiction in rats. I: Withdrawal syndrome after repeated administration of morphine. *Folia Pharmacol. Jpn.* **54**, 120, 1958.
11. Martin, W.R., Wikler, A., Eades, C.G. and Pescor, F.T.: Tolerance to and physical dependence in rats. *Psychopharmacologia* **4**, 247-260, 1963.
12. Nakamura, H., Ishii, K., Yokoyama, Y., Motoyoshi, S. and Shimizu, M.: A method for evaluating physical dependence liability of analgesic in rats. *Folia Pharmacol. Jpn.* **71**, 105, 1975.
13. Kosterlitz, H.W. and Watt, A.J.: Kinetic parameters of narcotic agonists and antagonists with particular reference to N-allylno roxymorphone (Naloxone). *Br. J. Pharmacol. Chemother.* **33**, 266-276, 1968.
14. Litchfield, J.T.Jr. and Wilcoxon, Y.: A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* **96**, 99-113, 1949.
15. Pearl, J. and Harris, L.S.: Inhibition of writhing by narcotic antagonists. *J. Pharmacol. Exp. Ther.* **154**, 319-323, 1966.
16. Blumberg, H., Wolf, P.S. and Dayton, H.B.: Use of writhing test for evaluating analgesic activity of narcotic antagonists. *Proc. Soc. Exp. Biol. Med.* **118**, 736-766, 1965.
17. Pircio, A.W. and Gyls, J.A.: Oxilorphan (L-N-cyclopropylmethyl-3,14-dihydroxymorphinan): A new synthetic narcotic antagonist. *J. Pharmacol. Exp. Ther.* **193**, 23-34, 1975.
18. Cowan, A., Lewis, J.W. and Macfarlane, I.R.: Agonist and antagonist properties of buprenorphine, a new antinociceptive agent. *Br. J. Pharmacol.* **60**, 537-547, 1977.
19. Hirose, K., Matsushita, A., Kojima, Y., Eigyo, M., Joyama, H., Shiomi, T., Tsukinoki, Y., Hatakeyama, H., Matsubara, K. and Kawasaki, K.: The pharmacology of 20681-S, and 20682-S, 6-oxo-N-cyclopropylmethylmorphinans, as narcotic antagonist analgesics. *Arch. Int. Pharmacodyn.* **241**, 79-91, 1979.
20. Nakamura, H., Ishii, K., Yokoyama, Y., Motoyoshi, S., Natsuka, K. and Shimizu, M.: Analgesic and other pharmacological activities of a new narcotic antagonist analgesic (-)-1-(3-methyl-2-butenyl)-4-[2-(3-hydroxyphenyl)-1-phenylethyl]-piperazine and its enantiomorph in experimental animals. *J. Pharm. Pharmacol.* **32**, 635-642, 1980.
21. Kosterlitz, H.W., Lord, J.A.H. and Watt, A.J.: Morphine receptor in the myenteric plexus of the guinea-pig ileum. In *Agonist and Antagonist Actions of Narcotic Analgesic Drugs*, Macmillan, London, PP. 45-61, 1972.
22. Hutchinson, M., Kosterlitz, H.W., Leslie, F.M. and Waterfield, A.A.: Assessment in the guinea-pig ileum and mouse vas deferens of benzomorphans which have strong antinociceptive activity but do not substitute for morphine in the dependent monkey. *Br. J. Pharmacol.* **55**, 541-546, 1975.
23. Hughes, J., Smith, T., Morgan, B. and Fothergill, L.: Purification and properties of enkephalin-the possible endogenous ligand for the morphine receptor. *Life Sci.* **16**, 1753-1756, 1975.
24. Ward, S.J., Metcalf, G. and Rees, J.M.: The comparative pharmacology of morphine, ketocyclazocine and 2-hydroxy-5,9-dimethyl-2-allyl-6,7-benzomorphan in rodents (Proceedings). *J. Pharm. Pharmacol.* **29**, Suppl., 54P, 1977.
25. Akera, T. and Brody, T.M.: The addiction cycle to narcotics in the rat and its relation to catecholamines. *Biochem. Pharmacol.* **17**, 675-688, 1968.
26. Schaumann, W.: The paralyzing action of morphine on the guinea-pig ileum. *Br. J. Pharm.*

- macol.* **10**, 456-461, 1955.
27. Henderson, G., Hughes, J. and Kosterlitz, H.W.: A new example of a morphine sensitive neuro-effector junction: adrenergic transmission in the mouse vas deferens. *Br. J. Pharmacol.* **46**, 764-766, 1972.
 28. Kosterlitz, H.W., Leslie, F.M. and Waterfield, A.A.: Narcotic agonist and antagonist potencies of a homologous series of N-alkyl-norketobemidones measured by the guinea pig ileum and mouse vas deferens method. *J. Pharm. Pharmacol.* **27**, 73-78, 1975.
 29. Hughes, J., Kosterlitz, H.W. and Leslie, F.M.: Effect of morphine on adrenergic transmission in the mouse vas deferens. Assessment of agonist and antagonist potencies of narcotic analgesics. *Br. J. Pharmacol.* **53**, 371-381, 1975.
 30. Schulz, R., Cartwright C. and Goldstein, A.: Reversibility of morphine tolerance and dependence in guinea pig brain and myenteric plexus. *Nature* **251**, 329-331, 1974.
 31. Takagi, H., Shiomi, H., Ueda, H. and Amano, H.: Morphine-like analgesia by a new dipeptide, L-tyrosyl-L-arginine Kyotorphin and its analogue. *Eur. J. Pharmacol.* **55**, 109-111, 1979.
 32. Takagi, H., Shiomi, H., Ueda, H. and Amano, H.: A novel analgesic dipeptide from bovine brain is a possible met-enkephalin releaser. *Nature* **282**, No. 5737, 410-412, 1979.
 33. Kaneto, H. and Watanabe, J.: Tolerance to and dependence on opioids in isolated longitudinal muscle of guinea pig ileum-validity of the muscle preparation as a receptor model for opioids. *Folia Pharmacol. Jpn.* **76**, 347-354, 1980.
 34. Wuster, M. and Herz, A.: Opiate agonist action of antidiarrheal agents in vitro and in vivo-Findings in support for selective action. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **301**, 187-194, 1978.