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HZ2, a Selective Kappa-Opioid Agonist

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INTRODUCTION

The effects of opioid drugs are mediated by the three major types of receptors, the μ, δ, and κ opioid receptors. The pharmacological profile of an individual opioid depends on, among other factors, the particular population of receptor types that is activated by the drug. Many compounds with a predominant κ opioid receptor affinity have been reported (7,11,25,28,69,70,32,35), some of which have a long history of clinical use, such as pentazocine and butorphanol. Most of them have only limited κ-binding selectivity, however, and, in addition, have a mixed agonist-antagonist action profile. This is typical of all κ-opioid analgesics used clinically so far.

Within the last 20 years there has been a substantial effort to develop pure and selective κ opioid agonists for pain treatment. Many compounds of this type have been discovered and subjected to intensive pharmacological characterization. Surprisingly, no compound has yet reached therapeutic application.

It has been found in animals that selective κ opioid receptor agonists produce antinociceptive effects without causing the undesirable effects of μ-opioids, such as respiratory depression (35) and severe inhibition of gastrointestinal transit (64,31,25). They appear not to cause emesis or morphine-like physical dependence (69,70,35). Moreover, selective κ opioid receptor agonists do not cause cross-tolerance to morphine (69,70.32). However, the stimulation of the κ opioid receptor is associated with some undesirable effects, such as sedation and diuresis (35,15,68). Especially the benzomorphan class elicits dysphoric states in humans (46). In animals the κ opioid receptor agonist U50,488H was reported to induce conditioned place and taste aversion (58) which is likewise an indication of unpleasant psychotropic properties.

The dysphoric and hallucinatory side effects seem to be the most important drawbacks of the κ opioid agonists and are frequently the reason why newly developed compounds are not used therapeutically (2). However, strong psychotropic side effects

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are not generally associated with the use of κ compounds. It seems that they depend more on individual structural features than being strictly correlated to the degree of κ receptor interaction. Therefore, molecules with a new and atypical structure might provide the best opportunity to detect compounds with a reasonable dissociation of these properties from analgesia. In comparison with the wide spectrum of undesirable side effects of the μ-receptor agonists such as morphine, these new κ agonists would be an attractive alternative for pain treatment.

A new and structurally unique group of compounds with remarkable opioid receptor affinity are the 9-phenyl substituted 3-aza- and 3,7-diazabicyclo[3.3.1]nonanones (5). Compounds with hydroxyl groups on the phenyl ring demonstrate a particularly high receptor affinity and strong antinociceptive potency (43,55). These molecules contain a 4-phenylpiperidine moiety (6), which is the dominant structure of the highly potent and clinically used μ-opioids of the pethidine and prodine groups. In contrast, the 2,4-aryl-substituted 3,7-diazabicyclo[3.3.1.]nonan-9-one-1,5-diesters, discussed here, are devoid of the 4-phenyl piperidine moiety and are, thus, unfamiliar structures for opioid receptor interactions.

Preliminary screening with the phenylquinone writhing test in mice revealed an opioid-type analgesic activity in these compounds, which could be antagonized by naloxone. Interestingly, no Straub tail reaction was observed (58). These results prompted us to perform radioligand binding studies, which for some compounds such as dimethyl 7-methyl-2,4-di-2-pyridyl-3,7-diazabicyclo[3.3.1]nonan-9-one-1,5-dicarboxylate [HZ1] showed a high affinity and selectivity for the κ opioid receptor (4,5).

These promising pharmacological findings initiated an intensive search for high-affinity κ agonists within this group of diazabicycles. The most interesting derivative is the N3-methylated dimethyl-2,4-di-2-pyridyl-3,7-diazabicyclo[3.3.1]nonan-9-one-1,5-diester, denoted as HZ2 (Fig. 1). The κ-receptor affinity and selectivity of HZ2 are comparable to those of HZ1. In contrast to HZ1, HZ2 showed a high oral availability in different animal species. Therefore, this compound was selected for the extensive pharmacological characterization that is presented here.

CHEMISTRY

Synthesis and Physicochemical Properties of HZ2

The diazabicyclo[3.3.1]nonan-9-one skeleton can be built up in a two-step Mannich reaction. In the first step, by refluxing in ethanol or methanol 1 mol of glutaric acid ester, 2 moles of a corresponding arylaldehyde, and 1 mol of ammonia or methylamine, a piperidone ring is formed, which can be further cyclicized using paraformaldehyde and methylamine to give the diazabicyclus (23,56). According to the synthesis pathway, the compounds are not stable in hot acid solution, such as 1 M hydrochloric acid. The higher substituted piperidone ring decomposes and can be isolated as the new product *N*-methyl-4-oxo-piperidone-3,5-dicarboxylate (23).

HZ2 is characterized by four pK_a values, 11.0 ± 0.1 and 8.7 ± 0.3 for the nitrogens in the heterocyclic skeleton, and 6.1 ± 0.1 and 5.2 ± 0.3 for the pyridine nitrogens (60). The strong basicity of the nitrogens in positions 3 and 7 causes a high degree of

Fig. 1. Chemical structure of HZ2.

protonation under physiological conditions (approximately 80% double protonation at pH 7.4). Interestingly, HZ2 still exhibits a certain degree of lipophilicity at pH 7.4 (log $D = 0.042$), indicating that the substance should be able to cross the blood-brain barrier. Due to its polar character, the compound has a low protein binding capacity. The degree of protein binding amounts to about 20% as determined by means of continuous ultrafiltration (U. Holzgrabe, G. Zlotos, P. Nickel, preliminary results).

Structure-Activity Relationships of Diazabicyclononanones

Since the molecular structure of HZ2 differs from all other κ ligands, it would be interesting to discover whether the physicochemical properties conform with the known ligands for the κ receptor. Therefore, an extended series of various compounds consisting of piperidone-3,5-diester, 3-oxa-7-aza-, and 3,7-diazabicyclo[3.3.1]nonan-9-one-1,5-diesters was compared with the κ-agonistic arylacetamide derivatives, U-50488, U-69593, a benzothienyl derivative of U-62066, CI-977 (enandoline), stereoisomers of EMD-61753, an isoquinoline derivative, and ketocyclazocine, by means of molecular modeling and NMR-spectroscopic methods (5). It was found that the chair-boat conformation and the protonation at the nitrogen atom N7 of the diazabicycles was the pharmacophoric conformation of this assembly of compounds. Comparison of the spatial arrangements, as well as the molecular, electrostatic, hydrophobic, and hydrogen-binding potentials of the above-mentioned κ-selective ligands, revealed a model of structure-activity relationship of agonists at the κ receptor. In this model the spatial arrangement of the pharmacophoric elements is characterized by an almost parallel orientation of a carbonyl group and a protonated NH function in conjunction with at least one aromatic ring (Fig. 2). A second aromatic ring at a distance

Fig. 2. Stereochemical conformation of HZ2. N_7-protonated chair-chair and chair-boat conformations. Arrows mark the most important pharmacophoric elements.

of 8 Å from the first benzene ring (distances of centroids) seems to enhance the affinity to the κ receptor.

PHARMACOLOGY

All animal testing was performed in accordance with the recommendations and policies of the International Association for the Study of Pain and the national law on the care of animals in experiments, German Animal Welfare Law. All study protocols were approved by the local government committee for animal research, which is also an ethics committee.

Biochemical Investigations

Specific binding of radioactive ligands for opioid receptor subtypes was investigated in *in vitro* experiments. The radioligands for μ, δ, and κ sites were [^{3}H]naloxone, [^{3}H]Cl-DPDPE, and [^{3}H]CI 977. The methods for the preparation of the membrane particles to perform receptor studies are given in a recently published paper (19).

Table 1 summarizes K_i values for HZ2 and reference compounds. HZ2 was found to be a κ-selective opioid. The specificity is quite high, i.e., the μ/κ quotient is in the range of at least two orders of magnitude. The reference agonists U-69593 and U-50488 are likewise κ-selective. Their absolute affinity to this receptor subtype is higher than that of HZ2 by a factor of three to four. In contrast, the reference compound bremazocine is non-subtype selective, showing a high affinity for all three opioid receptors.

Besides the opioid receptor interaction, the affinity of HZ2 to additional central nervous system target sites was investigated (Table 2). No relevant interaction with these receptors, uptake sites, ion channels, or enzyme system was detected.

TABLE 1. K_i values at μ-, δ-, and κ opioid receptors in membrane preparations of the rat brain

	Opioid Receptors K_i (μM ± S.E.M.)		
Substances	κ	μ	δ
HZ2=	0.015 ± 0.004	> 1=	> 10
U-50488=	0.0053 ± 0.001 =	1.6 ± 0.4 =	2.6 ± 0.7
U-69593=	0.0035 ± 0.00054 =	5.4 ± 1.0 =	1.4 ± 0.4
Bremazocine=	0.00021 ± 0.00003=	0.0006 ± 0.0001=	0.00051 ± 0.00011
Morphine=	0.17 ± 0.02 =	0.0022 ± 0.001 =	0.08 ± 0.0001

TABLE 2. Interaction of HZ2 wit the target sites listed below

Assay	Ligand/Substrate	Source of biological material	Test concentration	Inhibition, %
DA – D_1 receptor	[^{3}H]SCH 23390	Rat brain, Corpus striatum	10^{-4} M	< 10
Muscarinic receptor	[^{3}H]*N*-methyl scopolamine	Rat brain	10^{-6} M	10.0
α_1 Adrenoceptor	[^{3}H]prazosine	Rat brain, cortex	10^{-5} M	25.5
5-HT_{1A} receptor	[^{3}H]8-OH-DPAT	Rat brain	10^{-5} M	23.5
5-HT_2 receptor	[^{3}H]ketanserine	Rat brain, cortex	10^{-5} M	< 10
5-HT_3 receptor	[^{3}H]-BRL 43.694	Rat brain	10^{-5} M	< 10
NMDA receptor (PCP site)	[^{3}H]MK801	Rat brain	10^{-5} M	< 10
NMDA receptor (polyamine site)	[^{3}H]ifenprodil	Rat brain, cortex	10^{-5} M	< 10
Nicotinic ACh receptor	[^{3}H]cytisine	Rat brain	10^{-4} M	< 10
Sigma 1 receptor	(+)[^{3}H]pentazocine	Guinea pig brain	10^{-4} M	28.9
L-Ca^{2+} channel (phenylalkylamine site)	[*N*-Methyl-^{3}H]verapamil	Rat brain, cortex	10^{-5} M	17.3
L-Ca^{2+} channel (benzothiazepine site)	[^{3}H]Cis(+)Diltiazem	Rat brain, cortex	10^{-5} M	19.3
Na^+ channel (binding site 2)	[^{3}H]BTX	Rat brain, cortex	10^{-5} M	12.2
ACh-esterase	Acetyl-S-choline	Electric eel	10^{-4} M	19.3
Choline uptake	[^{3}H]Choline	Rat brain, Corpus striatum	10^{-5} M	< 10
NA uptake	[^{3}H]-1-Norepinephrine	Rat hypothalamus, Synaptosomes	10^{-6} M	< 10
DA uptake	[^{3}H] Dopamine	Rat brain, Corpus striatum	10^{-6} M	< 10

Abbreviations: DA, dopamine; 5-HT, serotonin; NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine; ACh, acetylcholine; NA, noradrenaline.

Opioid-Receptor Interaction in Isolated Organs

The effect of HZ2 on electrically evoked twitch contraction was studied in guinea pig isolated ileum. HZ2 inhibited twitch contraction in a dose-dependent manner with an IC_{50} of $5.91 \pm 0.79 \times 10^{-7}$ mol/l. At 10^{-5} mol/l, HZ2 induced a 75% inhibition of the baseline contraction. This response was antagonized only with a high dose of the relatively non-selective opioid antagonist naloxone (100 µmol/l). In contrast, morphine had an IC_{50} of 0.1 ± 0.004 µmol/l in this model, and was completely antagonized with naloxone (1 µmol/l), indicating involvement of different opioid receptor in the effects of HZ2 and morphine.

The rabbit vas deferens preparation (44) was electrically stimulated and the resultant contractions were recorded isometrically. Cumulative concentration-response curves were obtained for HZ2 with an IC_{50} of $8.23 \pm 9.23 \times 10^{-6}$ mol/l. Nor-binaltorphine 1 µmol/l, a highly selective κ-opioid antagonist (63), completely inhibited the twitch contraction of HZ2, confirming the selectivity of HZ2 for κ opioid receptors.

Acute Antinociception

Acute antinociceptive tests have been performed using thermal (tail-flick, hot-plate) (12,16), chemical (phenylquinone writhing test) (27), or electrical (tooth pulp stimulation) (47) stimuli in rats, mice, and rabbits (Table 3). In all tests HZ2 produced dose-related antinociception. The maximum attainable antinociceptive response was obtained in each test, indicating full agonistic activity of the compound. The peak effects were attained within 10 to 40 min after intravenous (i.v.) and 30 to 90 min after oral (p.o.) application.

The antinociceptive ED_{50} values (and 95% confidence intervals) in mice after intravenous administration ranged from 0.33 (0.23 to 0.43) mg/kg in the phenylquinone-induced writhing test to 3.18 (2.04 to 4.97) mg/kg in the 48°C hot-plate test. HZ2 also showed high potency in the tail-flick test in mice with an ED_{50} of 2.23 (1.80 to 2.71) mg/kg (Table 3).

Interestingly, after intravenous application in mice, the compound revealed an unusually long duration of action (Fig. 3). Antinociception persisted for more than 7 h after administration. This effect seems to be species dependent, since in the rat the duration of action was considerably shorter (data not shown). Moreover, the potency of HZ2 in the rat seems to be lower, since after intravenous application the compound was about three times less potent, and after oral application about six times less potent than in mice.

The oral availability of HZ2 can be estimated by comparing oral and intravenous ED_{50} values. Table 3 shows that in contrast to morphine HZ2 has a high oral availability in both, mice and rats. This is very pronounced in the tail-flick test in mice, where the intravenous: oral potency ratio is about 0.5 for HZ2, compared with about 0.05 for morphine.

One of the most reliable antinociceptive tests in animals is the electrically evoked tooth pulp stimulation in rabbits (47). Opioids and other predominantly centrally acting strong analgesics are most effective in this test. HZ2 elevated the threshold for elicitation of the licking and chewing response with in ED_{50} of 0.64 (0.42 to 0.95)

mg/kg i.v.. In comparison to morphine, HZ2 is noticeably potent in this test. The potency difference reaches a factor of about four in favor of HZ2. In other pain models HZ2 is slightly less than or maximally as potent as morphine.

The effect of naloxone on HZ2 antinociception was examined in the writhing test (Fig. 4). HZ2 or morphine (as reference) was given orally 30 min, and naloxone intravenously (in escalating dosages) 5 min before intraperitoneal (i.p.) administration of phenylquinone. The dose of both agonists used in this test completely inhibited the writhing reactions. We evaluated the antagonistic dose (AD_{50}) of naloxone, which reduced the writhing response by 50% in comparison to the controls (untreated). Naloxone antagonized the writhing inhibition of HZ2 and morphine in a dose-dependent manner. The AD_{50} obtained for the HZ2/naloxone interaction was about five times higher than the AD_{50} for the morphine/naloxone interaction, indicating a lower potency of naloxone for displacing the κ opioid agonist HZ2 from the receptor.

Inflammatory and Persistent Pain

The effect of HZ2 has been studied in two models of inflammatory and persistent pain in rats, the Randall-Selitto test (51) and the formalin test (14). The Randall-Selitto test is a model of acute transient inflammatory pain, which is induced by local application of brewer's yeast into a hindpaw, leading to an increased sensitivity towards mechanical pressure exerted on the paw. The formalin test is a frequently used model for persistent pain, which is mostly focused on the early phase of the chronification process. Formalin injection into the paw leads to a characteristic biphasic behavioral pain response. Both phases can be blocked to different degrees by several pain inhibitory mechanisms.

Systemic application of HZ2 led to a dose-dependent antinociceptive effect in the Randall-Selitto test with an ED_{50} of 27.7 (23 to 34.6) mg/kg i.p. To prove a possible central action of HZ2, the compound was tested by intrathecal administration. As with

TABLE 3. HZ2-induced antinociception in mice, rats, and rabbits

			ED_{50} (mg/kg)	
Test=	Species=	Route	HZ2=	Morphine
Abdominal constriction	mouse=	i.v.=	0.33 (0.23 – 0.43)=	0.33 (0.19 – 0.51)
		p.o.=	2.49 (1.48 – 3.61)=	4.70 (3.46 – 6.33)
Hot-plate (48°C)	mouse=	i.v.=	3.18 (2.04 – 4.97)=	1.33 (1.13 – 1.55)
		i.p.=	16.5 (12.9 – 20.2) =	4.72 (3.75 – 5.77)
Tail-flick	mouse=	i.v.=	2.33 (1.80 – 2.71)=	1.44 (1.10 -1.79)
		p.o.=	4.88 (3.80 – 6.57)=	26.1 (21.8 – 30.5)
Tail-flick	rat=	i.v.=	5.49 (4.69 – 6.51)=	1.11 (0.78 – 1.47)
		p.o.=	24.0 (20.0 – 31.5) =	32.5 (22.6 – 43.3)
Tooth pulp stimulation	rabbit=	i.v.=	0.64 (0.42 – 0.95)=	2.28 (1.80 – 2.86)

Abbreviations: i.v., intravenous; p.o. oral; i.p., intraperitoneal. All values are expressed as the ED_{50} (95% confidence intervals) at time of peak effect. Morphine is included for comparison.

Fig. 3. HZ2 and morphine dose-response curves after intravenous administration in the tail-flick test in mice. Each point on the graph represents the mean ± S.E.M., $n = 10$ animals per group. % MPE (maximal possible effect) was determined as follows:

$$\%\ \mathrm{MPE} = \frac{\text{test latency} - \text{predrug latency}}{\text{cutoff time} - \text{predrug latency}} \times 100$$

Fig. 4. Antagonism by naloxone of the antinociceptive effects of an ED_{100} dose of HZ2 and morphine in the phenylquinone writhing test. The agonists were given orally 30 min and naloxone intravenously (in escalating dosages) 5 min before phenylquinone was administered intraperitoneally. The antagonistic dose (AD_{50}) was determined. Each column represents the mean response rate with S.E.M., $n = 10$ animals per group.

systemic administration the central route showed good efficacy with an ED_{50} of 23.3 (16.9 to 29.4) μg/animal. This central effect was partially antagonized (32%) by coadministration of the κ-specific antagonist nor-binaltorphimine (8 μg/animal), whereas the μ-antagonist naloxone had no effect.

HZ2 showed antinociceptive activity in the formalin test. At a dose producing a half-maximal inhibition in the Randall–Selitto test (21.5 mg/kg i.p.), HZ2 inhibited both the early and late phase of the formalin pain reaction. The pain score, which is determined by three behavioral modalities (lifting, flinching, and licking/biting of the formalin injected paw), was reduced in the first phase by 47% and in the second phase by 60%. All three behavioral parameters were reduced to a similar degree by HZ2.

Other Opioid Related Effects

The physical dependence potential of HZ2 was investigated in mice according to the method of Saelens et al. (54), multiple drug doses were administered over 2 d, followed by naloxone-precipitated withdrawal. After administration of HZ2 in an escalating dose scheme up to a final dose of 21.5 mg/kg i.p., no withdrawal behavior (jumping) was induced by naloxone. Naloxone was used at a high dose of 30 mg/kg i.p. so that κ-induced effects could be antagonized. Further behavioral tests in order to study the motivational properties of HZ2, such as place-preference or self-application tests, were not performed.

From the literature it is known that κ agonists induce conditioned place and taste aversion in animals (57). In addition, it is reported that treatment with the κ opioid receptor agonists U69593 and U50,488H abolishes cocaine-induced behavioral sensitization (26), probably by normalizing the cocaine-induced increases in basal levels of dopamine overflow in the nucleus accumbens (37). There is also evidence that κ opioid receptors play a role in the mediation of drug-induced reward. It has been reported that κ opioid receptor agonists attenuate the preference for both morphine (21,42) and cocaine (9,62) in a conditioned place preference paradigm.

The influence of HZ2 on the gastrointestinal tract was investigated in two models, the charcoal intestinal passage (39) and prostaglandin E2 (PGE2)-induced diarrhea (53). The charcoal test, reflecting the transit time, shows the influence on the upper part of the gastrointestinal tract up to the cecum. Additionally, PGE2 (100 μg/kg i.p.)-induced diarrhea in mice was used to test antisecretory and antidiarrheogenic properties. HZ2 inhibited charcoal passage with an ED_{25} of 2.15 (1.47–3.12) mg/kg i.p. and PGE2-induced diarrhea with an ED_{50} of 9.20 (6.34–13.4) mg/kg i.p. More than 50% inhibition in the charcoal passage could not be achieved because of toxic side effects at doses from 31.5 mg/kg i.p. upwards. HZ2 is approximately 3-fold less potent than morphine in the PGE2-induced diarrhea and inhibition of charcoal passage.

A possible emetic activity of HZ2 was investigated in the ferret. This model is frequently used to reveal the emetic potential of opioids (65), since centrally mediated emetic properties of opioids as well as their antiemetic potential in the higher dose ranges are in ferrets similar to those in humans. In addition, the anatomical and physiological characteristics of the gut of the ferret are similar to those of the human gut (36). HZ2 induced a dose-dependent emetic activity, including retching and vomiting in the dose range of 0.464 to 10.0 mg/kg i.p. Higher doses were associated with

an earlier onset of the vomiting periods. Unlike morphine, HZ2 does not seem to decrease emesis at higher doses, since up to the highest nontoxic dose (10 mg/kg i.p.), there was no reduction of emesis. Naloxone pretreatment completely antagonized the emetic effect of HZ2.

HZ2 caused a dose-dependent suppression of spontaneous locomotor activity (sedation) in mice at doses close to those required to produce antinociception. In the hole-board test (3), exploration motility was decreased with an ED_{50} of 4.27 (3.27 to 6.28) mg/kg i.p. Confirming the sedative effect seen in motility tests, HZ2 potentiated pentobarbital sleeping time (17) in mice with an ED_{200} of 5.14 (3.99 to 6.38) mg/kg i.p. Additionally, a decrease of motor coordination and tracking performance was seen in the rotarod test in mice. The motor performance was significantly impaired by HZ2 at doses $\geq$ 14.7 mg/kg i.p. An ED_{50} value of 17.0 (1.3. 3 to 20.3) mg/kg i.p. was calculated.

κ-Opioid receptors are involved in the regulation of diuresis (24,52). Agonists at κ opioid receptors increase diuresis most probably via inhibition of the release of antidiuretic hormone (ADH) from the pituitary gland (34). By intravenous administration, HZ2, 0.1 mg/kg, did not affect urine volume or excretion of electrolytes (Na^+, Cl^-, K^+) in conscious rats. In the dose range from 0.464 to 10 mg/kg, however, HZ2 produced a large, not strictly dose-dependent, increase in diuresis and saluresis. This effect lasted for about 2 h. This diuretic activity of HZ2, occurring in the antinociceptive dose range, is consistent with a κ opioid receptor-mediated regulation of fluid excretion.

By intravenous administration HZ2 at 0.464 to 10 mg/kg did not influence the spontaneous respiratory rate in conscious rats. The increase in respiratory rate provoked by admixture of CO_2 (final concentration 5%) to the inspiration air was not reduced by intravenous HZ2 at doses up to 2.15 mg/kg; a reduction of CO_2-stimulated increase in breathing frequency occurred after HZ2, 4.64 and 10.0 mg/kg i.v., when the respiratory rate rose from 123 breaths per min at baseline (before drugs and before CO_2) to 169 and 176 breaths per min, respectively. In contrast, after treatment with the vehicle, the CO_2-stimulated respiratory rate increased to 213 breaths per min. The modest effect of HZ2 on respiration is in line with the more common finding that κ opioid receptor agonists have limited effects on respiratory function (38,18).

Cardiovascular Effects

In isolated, spontaneously beating guinea pig atrial preparations, HZ2 induced a concentration-dependent decrease of the beating frequency. The half-maximal concentration (EC_{50} value with 95% confidence limits) for this negative chronotropic effect of HZ2 was 137 (119–158) μM. In isolated, electrically driven papillary muscles from guinea pigs, HZ2 exerted a negative inotropic effect with an EC_{50} value of 38 (24–52) μM. The negative chronotropic and inotropic effects of HZ2 could easily be reversed with full recovery of contractile function after washout. Heart rate in conscious rats was reduced in a dose-dependent manner by intravenous injections of 1.0 to 10 mg/kg HZ2, with a maximum decrease of about 20%. Blood pressure in these normotensive rats was affected at the dose of 10 mg/kg HZ2, which also produced extrasystoles in about half of the animals. In urethane-anesthetized mice, HZ2 (1.0 to 4.64 mg/kg i.v.)

exerted a dose-dependent antiarrhythmic effect, demonstrated by an increase in the arrhythmogenic dose of aconitine from 61 μg/kg in vehicle-treated controls to 109 μg/kg after pretreatment with the highest dose of HZ2. The antiarrhythmic potency of HZ2 was 3- to 4-fold lower than that of quinidine sulfate. The cardiovascular *in vitro* (negative chronotropy and inotropy) and *in vivo* (bradycardia, antiarrhythmic effect) effects of HZ2 are best explained by an unspecific membrane-stabilizing effect at doses above the antinociceptive dose range.

Toxicology

Toxicological investigations of HZ2 were carried out in rats and mice after single dose administration, using oral, intravenous, and intraperitoneal routes. The dose levels of HZ2 ranged from 14.7 to 681 mg/kg body weight. Four animals were used per dose level. Signs of toxicity were assessed following a modified protocol originally described by Irwin (29). Behavior and general condition of the animals were observed for up to 6 h following drug administration during a total observation period of 7 d. The behavioral profile (awareness, mood, motor activity), neurological symptoms (central excitation, motor coordination, muscle tone, reflexes), autonomic reactions, and general toxicological symptoms were monitored by evaluating up to 32 different parameters.

The results of the toxicological investigations are summarized in Table 4. The main symptoms were irregular respiration, reduced spontaneous activity, ventral recumbency and reduced body weight gain. Seizures were seen only at dose levels near the lethal range (mouse: 21.5 mg/kg i.v. and 681 mg/kg p.o.; rat: 464 mg/kg p.o.).

The pattern of side effects seen with HZ2 is in agreement with findings reported for other selective κ opioid agonists. In particular, locomotor impairment is described as typical of κ agonists (35,22,41).

In our investigations, seizures were not seen after HZ2 except at doses near the lethal range. Similar observations were made with other κ agonists. On the contrary, even an inhibitory effect on experimental limbic seizures is described for some κ

TABLE 4. Toxicological findings after single dose administration of HZ2 in rats and mice

	Mouse (mg/kg)=			Rat (mg/kg)		
	i.v.=	i.p.=	p.o.=	i.v.=	i.p.=	p.o.
Dose range tested (mg/kg)	14.7–31.6	46.4–681	100–681=	21.5–46.4	316–681	147–681
Reduced spontaneous activity	14.7=	46.4=	100=	21.5=	316=	147
Irregular respiratory activity	14.7=	46.4=	100=	21.5=	316=	147
Ventral recumbency	14.7=	68.1=	147=	31.6=	464=	147
Convulsions	21.5=	–=	681=	–=	–=	464
Reduction of body weight	14.7=	46.4=	100=	21.5=	316=	–
LD_{50} range (approx.)	26.1–31.6=	464–681	464–681=	31.6–46.4	464–681	464–681

Abbreviations: i.v., intravenous; i.p., intraperitoneal; p.o., oral. Values are the individual test doses at which the indicated symptoms were observed.

agonists (49,66,33). In summary, HZ2 revealed no specific toxicological findings unknown for κ agonists.

SUMMARY AND CONCLUSIONS

We analyzed the pharmacological profile of HZ2, a selective κ opioid receptor agonist, with respect to its potential therapeutic value as an analgesic drug. *In vitro*, HZ2 exhibits selective affinity and high agonist potency at the κ receptor in opioid ligand binding and isolated organ assays. No relevant affinities to other targer sites were detected. In the *in vivo* rodent and rabbit nociception-related models induced either by physical, chemical, or thermal noxious stimuli, HZ2, following systemic administration, shows a strong antinociceptive activity comparable to morphine. In addition, HZ2 exerts a potent action in inflammatory and persistent pain shown in the Randall–Selitto and formalin tests, respectively.

An unusually long duration of action was seen in the mouse tail-flick test after intravenous or oral administration. Moreover, the compound shows a high oral availability, and in this respect is superior to morphine.

A further advantage of HZ2 in comparison to the κ opioid agonist is the absence of dependence liability and respiratory depression. Effects of κ agonists on gastrointestinal functions have been discussed in the literature with some controversy (69,70,35). Reasons for the discrepancies regarding the relative functional significance of opioid receptor types depend on the species investigated, the motility measure used, the time period covered by the experiment, or the gut region examined (30). Nevertheless, the direct comparison of HZ2 and morphine in the charcoal intestinal passage and PGE2-induced diarrhea tests in mice revealed that the constipational potential of HZ2 is approximately 3-fold less than that of morphine.

The sedation, diuresis, and saluresis produced by HZ2 at doses close to the antinociceptive dose are consistent with the expected profile for a κ receptor agonist (15,34,35,67,45). The dose-dependent emesis, also seen in the antinociceptive dose range, is not a typical κ receptor mediated effect, but may be related to the special chemical structure of the compound.

In conclusion, HZ2 exhibits strong antinociceptive properties with a potency comparable to morphine. HZ2 possesses a typical κ-agonistic profile, exhibiting a panel of positive and negative activities: it has no morphine-type physical dependence or respiratory depression, but does produce diuresis and sedation. Morphine and related opioids currently provide the mainstay of analgesic therapy for severe pain. κ-Opioid receptor agonists have been developed to avoid the adverse effects of morphine-like μ-selective analgesics while preserving their analgesic activity. With respect to its antinociceptive efficacy, the κ agonist HZ2 is comparable to morphine. However, the κ-receptor-specific side effects in combination with the drawbacks of κ agonists in clinical studies (induction of dysphoria or psychotomimesis) raise questions as to the therapeutic utility of this pharmacologically interesting compound.

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