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Analgesic and Acute Central Nervous System Side Effects of the Intravenously Administered Enkephalinase Inhibitor SCH 32615

RICHARD E. CHIPKIN[1] AND VICKI L. COFFIN

Schering-Plough Research, 60 Orange Street, Bloomfield, NJ 07003

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CHIPKIN, R. E. AND V. L. COFFIN. *Analgesic and acute central nervous system side effects of the intravenously administered enkephalinase inhibitor SCH 32615.* PHARMACOL BIOCHEM BEHAV **38**(1) 21–27, 1991.—The analgesic and acute central nervous system (CNS) side effect potential of the enkephalinase inhibitor SCH 32615 (N-[L-(-1-carboxy-2-phenyl)ethyl]-L-phenylalanine-β-alanine) were evaluated after IV administration to mice, rats and squirrel monkeys. In mice, SCH 32615 caused dose-related suppression of acetic acid-induced writhing (minimal effective dose, MED = 3 mg/kg IV). In rats, SCH 32615 produced dose-related increases in the response latencies in the yeast inflamed-paw test (MED = 10 mg/kg IV). In squirrel monkeys, using a new hot-water bath tail-flick test, SCH 32615 significantly prolonged the escape latencies (MED = 100 mg/kg IV). These results in primates are the first data showing an analgesic action of an enkephalinase inhibitor in a reflex model of pain. When measured for its CNS side effect potential, SCH 32615 had no significant effects in rats (up to 100 times its analgesically active doses) or in monkeys (up to three times). In the mouse, at doses 100 times its minimal effective dose, SCH 32615 produced brief convulsions; these lasted only a minute, resolved quickly, and did not cause lethality. In contrast, in rats and squirrel monkeys, the standard opioid analgesic morphine produced profound CNS side effects; this was particularly notable in monkeys, in which morphine's maximal analgesic effects were associated with near lethal respiratory depression. These data demonstrate that SCH 32615 produces selective analgesic actions and that its acute side effect liability is less than that seen with a clinically used standard.

Enkephalinase inhibitor SCH 32615 Analgesic

ENKEPHALIN is the common name given to either of two pentapeptides (TyrGlyGlyPheMet or TyrGlyGlyPheLeu). In the brain, their function has been proposed to be the control of nociceptive information processing. The rate limiting step in enkephalin's actions is its enzymatic hydrolysis at the glycinyl-phenylalanine amide bond. The enzyme responsible for this has several names, but is technically called neutral endopeptidase and has been designated by the Enzyme Commission as E.C.3.4.24.11. Since it appears as if the primary role of the enzyme in the central nervous system (CNS) is in the regulation of the opioid peptide enkephalin, it has also been given the trivial name of enkephalinase (Enk'ase)(2). Consistent with this, Enk'ase inhibitors have been shown to decrease the levels of the purported metabolite in the brain (i.e., TyrGlyGly) and to produce naloxone-reversible antinociceptive effects (7,8).

SCH 32615 (N-[L-(-1-carboxy-2-phenyl)ethyl]-L-phenylalanine-β-alanine) has been demonstrated to potently and selectively inhibit Enk'ase in vitro (1). However, its in vivo pharmacological actions have not previously been investigated. The following studies were designed to assess the analgesic actions of intravenously (IV) administered SCH 32615 in several animals models, to compare it with a known standard (i.e., morphine) and to determine the acute CNS side effects of the drug relative to its analgesic actions. The systemic route of administration was chosen since previous work from this lab had determined that the drug was not orally active (Chipkin, unpublished results). The present studies are of interest for two reasons. First, the pharmacology of an Enk'ase inhibitor across species has not been described previously. Second, no prior studies have confirmed an antinociceptive action of Enk'ase inhibitors in nonhuman primates.

METHOD

General

Male CF1 mice, 20–24 g, and male Sprague-Dawley rats, 100–120 g, were obtained from Charles River Breeding Labora-

[1]Requests for reprints should be addressed to Richard E. Chipkin at his present address: Schering-Plough Research, 2000 Galloping Hill Road, Kenilworth, NJ 07033.

tories, Wilmington, MA for use in all studies. Squirrel monkeys (0.8–1.2 kg) were obtained from Hazelton Labs (Cumberland, VA).

In the analgesic studies, the experimental conditions were designed to elicit a consistent, measurable response while minimizing stress to the animals. These experiments conform to the "Guidelines for Investigations of Experimental Pain in Conscious Animals" (9).

For all these studies, the SCH 32615 sample used was synthesized at Schering-Plough Corp. Morphine was purchased commercially (Penick Co.) and naloxone was a gift from Dupont Inc.

Mouse Acetic Acid-Induced Writhing Test

A modification of the method of Hendershot and Forsaith (4) was employed except that acetic acid rather than phenylquinone was used to evoke writhing (6). Groups of at least 10 mice were injected intraperitoneally with 0.6% aqueous acetic acid (10 ml/kg) at 15 minutes after the intravenous administration of the test drug or vehicle (volume of injection was 0.1 ml/injection/mouse, except for the dose of 1000 mg/kg IV for which the volume of injection was 0.3 ml/injection/mouse). The mice were then placed in a large observation beaker and the number of writhes were counted during a ten-minute period starting 3 minutes after the injection of acetic acid. A writhe was defined as a sequence of arching of the back, pelvic rotation, and hind limb extension. The data were analyzed by taking the number of writhes each drug-treated animal showed and dividing it by the mean number of writhes seen in the vehicle-treated animals. This fraction was then multiplied by 100% to calculate the percent inhibition of writhing for each animal. These values were used to determine mean values for each drug-treated group. The data were statistically analyzed using an analysis of variance and post hoc testing on each group was done using Dunnett's test.

Low-Yeast Rat Inflamed-Paw Test

The low-yeast rat inflamed-paw test was done as previously described (1). Briefly, groups of at least 5 rats were first tested for their response latencies to withdraw from pressure applied by an accelerating (20 mmHg/s) bullet-shaped piston to each rear paw. Subsequently, the right rear paw was inflamed by a subplantar injection (0.1 ml) of a 2.5% Brewer's yeast solution (w/v in distilled H_2O). Sixty minutes thereafter the response latencies were redetermined. Treatments were then administered intravenously (volume of injection was 1 ml/kg except at 300 which was 3 ml/kg) and at 30, 60 and 120 minutes thereafter the posttreatment latencies were determined. The data were analyzed using an unpaired *t*-test.

Squirrel Monkey Hot Water Tail-Flick Test

This test is a modification of the method of Dykstra and Woods (3). Briefly, squirrel monkeys were seated in restraining chairs with their tails hanging freely. The terminal 10 cm of the tail was shaved to permit all animals to be equally exposed to the noxious stimulus.

In this test, the animals were seated facing away from a styrofoam heat-retaining cylinder (diameter 7.5 cm) containing water heated to 50.4 ± 0.2°C. The shaved tail was placed in the container and the time until a "tail-flick" occurred was determined. A tail-flick is defined as a brisk, reflexive movement of the tail away from the bath. Animals were first screened on a separate day prior to any drug treatment to identify monkeys which responded to this temperature with a latency of less than 7 s over three consecutive trials. Animals meeting this criterion were subsequently used in the study.

On the test day, at least three baseline tail-flick latencies were determined. If the mean tail-flick latency remained below 7 s the animal continued in the experiment. Drugs were then injected intravenously (injection volumes were the same as for rats) and at various times thereafter the tail-flick latencies were redetermined (three tail-flicks were taken at each time point). The mean ± SE tail-flick latency was determined from each of the three values at each point. These were compared using an unpaired *t*-test to the control values. If there was a statistically significant ($p<0.05$) increase in the tail-flick latency, the animal was defined as "affected." The number of affected monkeys out of the total number of animals were used to construct dose-response curves. The minimal effective dose (MED) was defined as the dose at which >50% of the treated monkeys showed a significant increase of their tail-flick latencies.

To determine the sensitivity of the drug effect to opiate receptor blockade, the narcotic antagonist naloxone (5 mg/kg IV) was given 10 min prior to the intravenous injection of SCH 32615 (100 mg/kg). This dose of naloxone is known to fully reverse the effects of morphine (a standard opioid agonist analgesic) in other analgesic tests and this dose of SCH 32615 has been demonstrated to produce a maximum effect in the tail-flick test. Testing took place at 30, 60, 90, 120 and 180 min posttreatment with SCH 32615.

Effects on Behavior, Neurological and Autonomic Function

A modification of the method of Irwin (5) was used. Changes in behavior, neurological function and autonomic activity of mice and rats were evaluated at 15, 30, and 60 minutes after intravenous administration of vehicle, graded doses of SCH 32615 or morphine. All measurements were made using a semiquantitative scale in which a "normal" level of signs like spontaneous motor activity, pupil size, and alertness was assigned a score of "0" and scores of ±1, ±2, and ±3 indicated slight, moderate, and marked decreases from "normality." Signs not normally present (e.g., convulsions, tremors) were graded on a 1–3 scale.

The Irwin test was further modified for primates. In this case, 22 measures were used to evaluate the behavioral, neurological and autonomic effects of intravenously injected SCH 32615. The modified rating scale incorporated two significant changes from the one used for rodents. First, the scale was changed from 0 to ±3 to 0 to ±5; this was done to reflect the greater range of behaviors that monkeys display compared to rodents. Second, the test also included measures on interactions occurring between the investigator and the primate. This was done since monkeys show significantly different behavior when interacting with an observer than when that observer is at a distance. Ratings were done over the course of a 6h experiment. The data presented represents the maximum effect observed at any time during that period.

RESULTS

Mouse Acetic Acid-Induced Writhing Test

In this test, a dilute solution of acetic acid is injected intraperitoneally to induce a stereotypical writhing behavior. Drugs which are clinically analgesic decrease the number of writhes in a dose-related manner. Indeed, the simplicity and reliability of this test makes it the most commonly used assay for identifying active agents. The following study was done to evaluate the effects of SCH 32615 and compare them with the standard opioid analgesic morphine. The results from this study can be found in Fig. 1.

SCH 32615 caused a dose-related inhibition of acetic acid-in-

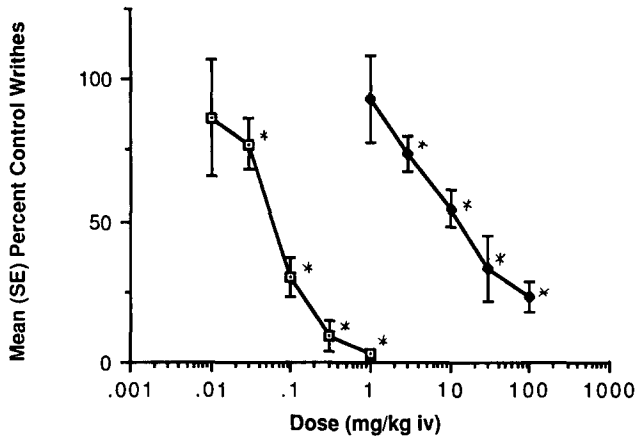

FIG. 1. Effect of intravenous SCH 32615 (●) or morphine (□) on acetic acid-induced writhing behavior in mice. N = 10/group. *Sig. different from vehicle, $p<0.05$.

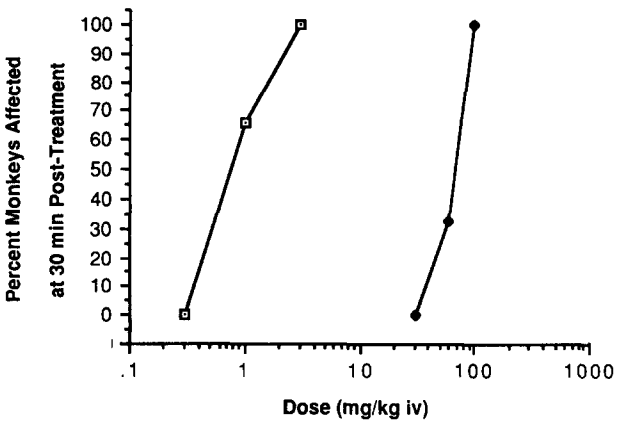

FIG. 2. Effect of intravenous SCH 32615 (●) or morphine (□) on the percent of animals affected using the squirrel monkey tail-flick test at 30 min posttreatment. The number of monkeys at each dose = 3. Vehicle had no significant effects on these animals when pre- and postinjection values were compared (mean ± SE preinjection = 3.0 ± 0.3 s vs. post-injection = 2.6 ± 0.3 s, NS).

duced writhing. The minimal effective dose (MED) for producing a statistically significant effect is 3 mg/kg. For purposes of comparison, the standard narcotic morphine also produced these same results, with an MED of 0.03 mg/kg.

Low-Yeast Rat Inflamed-Paw Test

In this assay, the right rear paw of a rat is injected in the subplantar region with a solution of Brewer's yeast to evoke a local inflammatory response. When pressure is applied to the paw, animals will attempt to escape from the noxious stimulus. Drugs known to produce analgesia clinically will increase the latencies to respond to the pressure. SCH 32615 was tested in this assay for its antinociceptive effects, with morphine included as a standard. The results from this study can be found in Table 1. SCH 32615 produced dose-related increases in the response latencies, with a MED of 10 mg/kg. The effect peaked at 30 min and lasted at least 120 min at doses >30 mg/kg. Morphine produced similar effects (MED = 0.003 mg/kg) as SCH 32615, and showed a comparable time course.

The response latencies of the noninflamed paw were also determined in this test. Drugs which are strong analgesics clinically (e.g., opiates) increase the noninflamed-paw latencies; mild analgesics (e.g., acetaminophen) have no effect on the noninflamed

TABLE 1

EFFECT OF SCH 32615, MORPHINE AND VEHICLE ON RESPONSE LATENCIES OF THE INFLAMED-PAW IN THE LOW YEAST RAT YEAST-PAW TEST

Treatment	Dose (mg/kg IV)	Mean (±SE) Response Latency (in s) of the Inflamed-Paw at Indicated Time: 30 Minutes	60 Minutes	120 Minutes
		Experiment 1		
Vehicle	—	4.28 ± 0.16	4.21 ± 0.15	3.99 ± 0.11
SCH 32615	3	4.57 ± 0.15	4.27 ± 0.12	4.16 ± 0.08
	10	5.46 ± 0.33*	5.41 ± 0.37*	4.29 ± 0.15
	30	7.82 ± 0.40*	7.37 ± 0.36*	4.85 ± 0.21*
	100	10.81 ± 0.27*	9.59 ± 0.22*	7.03 ± 0.24*
	300	13.79 ± 0.75*	12.82 ± 1.03*	8.13 ± 0.22*
		Experiment 2		
Vehicle	—	4.17 ± 0.14	4.12 ± 0.10	4.06 ± 0.10
Morphine	0.003	6.00 ± 0.36*	5.36 ± 0.23*	4.32 ± 0.09
	0.01	9.56 ± 0.21*	9.42 ± 0.43*	5.33 ± 0.30*
	0.03	14.19 ± 0.47*	13.69 ± 0.54*	8.69 ± 0.20*

*The data for SCH 32615 are the mean ± SE of three experiments merged together. The number of animals per group for vehicle and SCH 32615 at 3, 10, 30, 100 and 100 is as follows: 15, 10, 10, 15, 5 and 5. The number of rats used in Experiment 2 is 5/group.

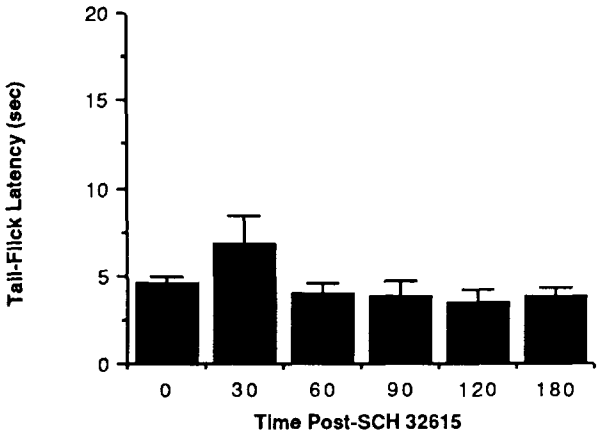

FIG. 3. Effect of naloxone (5 mg/kg SC) given 10 min prior to SCH 32615 (100 mg/kg IV) on tail-flick latencies in the hot-water bath tail-flick test in squirrel monkeys. The effect of SCH 32615 alone in this test is 17.9 ± 1.5 s. N = 3.

paw. It is, therefore, notable that SCH 32615 had significant effects on the noninflamed paw at 100 and 300 mg/kg (mean ± SE response latency for Vehicle = 6.96 ± 0.12 and for SCH 32615 at 100 and 300 mg/kg respectively = 8.06 ± 0.11 and 9.81 ± 0.83, $p<0.05$, t-test).

Squirrel Monkey Hot Water Tail-Flick Test

No previous studies have been able to identify an antinociceptive action of an Enk'ase inhibitor in a primate model. Therefore, a known assay was modified in an attempt to show an analgesic action of Enk'ase inhibitors in primates. In this test, the shaved end of a squirrel monkey's tail is immersed in water kept at a constant temperature (50.4 ± 0.2°C). After a period of time, the animal will make a brisk, reflexive movement of the tail out of the bath. This response is called a "tail-flick." Analgesic agents increase these response latencies. The effects of SCH 32615 were compared with a known pain reliever (i.e., morphine) in this test. The results can be seen in Fig. 2.

The untreated, baseline tail-flick latencies ranged between 2.5–5 s for all groups and did not differ significantly. At 30 min posttreatment, vehicle-treated animals had values of 3.0 ± 0.5 s; thus the injections themselves and/or the handling had no significant effects. In all cases, a 20-s cut-off time was used to avoid tissue damage to the animal.

SCH 32615 produced dose-related increases in the tail-flick latency in this test. The MED for this effect was 100 mg/kg (mean ± SE tail-flick latency at this dose = 17.9 ± 1.5 s). The standard narcotic morphine also produced a dose-related effect (MED = 1 mg/kg). Higher doses of morphine (3 mg/kg) produced greater analgesic effects (mean ± SE = 20 ± 0 s), but also caused severe respiratory depression that required rescue doses of naloxone to prevent lethality.

Since SCH 32615 is acting by potentiating the effects of the endogenous opioid peptide enkephalin, it follows that the analgesic effects observed behaviorally should be blocked by the narcotic antagonist naloxone. Indeed, this is the case for the analgesic effects of all Enk'ase inhibitors identified (2,8). Therefore, to determine if the effects of SCH 32615 were occurring via an opioid mechanism, the ability of naloxone to block the actions of SCH 32615 were investigated. The results (Fig. 3) show that a dose (100 mg/kg) of SCH 32615 previously shown to signifi-

TABLE 2

EFFECTS OF VEHICLE OR SCH 32615 ON BEHAVIORAL, NEUROLOGIC AND AUTONOMIC FUNCTION IN MICE

	Number of 6 Treated Mice that Manifested the Indicated Measurement* Following the Intravenous Injection			
		SCH 32615		
Measure	Vehicle	30 mg/kg	100 mg/kg	300 mg/kg
Passivity	0	0	0	0
Increased Reactivity Transfer	0	0	0	1
Decreased Pinna/Corneal Reflexes	0	0	0	0
Decreased Muscle Tone	0	0	0	0
Ataxia	0	0	0	0
Loss of Righting Reflex	0	0	0	0
Tremors/Twitches	0	0	0	0
Convulsions	0	0	0	6†
Miosis	0	0	0	0
Respiratory Difficulty	0	0	0	6‡
Lethality (24 hours)	0	0	0	0

*Results in this table are measurements made within the 0–15 minute period following injections. Incidence was considered positive when the indicated measurement was assigned a score of 2 or greater according to the rating system of Irwin (5). Results of measurements made at 30 and 60 minutes after injections (not presented here) were not different than those presented here for the vehicle-treated group.

†Clonic convulsions occurred immediately after injection and lasted about one minute.

‡Characterized only by increased respiratory rate occurring immediately after injection and lasting only about one minute.

TABLE 3

EFFECTS OF VEHICLE OR SCH 32615 OR MORPHINE ON BEHAVIOR, NEUROLOGIC AND AUTONOMIC FUNCTION IN RATS

	Number of 6 Treated Rats that Manifested the Indicated Measurement* at the Indicated Dose (mg/kg IV)						
		SCH 32615			Morphine		
Measure	Vehicle	100	300	1000	0.1	0.3	1.0
Passivity	0	0	1	3	0	0	0
Vocalization	0	0	0	4	0	3	6
Increased Reactivity Transfer	0	0	0	6	0	0	0
Decreased Pinna/Corneal Reflexes	0	0	0	0	0	0	0
Decreased Muscle Tone	0	0	0	0	0	1	0
Ataxia	0	0	0	6†	0	6†	6†
Loss of Righting Reflex	0	0	0	0	0	0	0
Ear Twitches	0	0	0	1	0	3	3
Convulsions	0	0	0	0	0	0	0
Miosis	0	0	0	0	0	0	0
Mydriasis	0	0	0	1	0	0	0
Exophthalmos and Staring	0	0	0	6‡	0	6‡	6‡
Respiratory Difficulty	0	0	0	0	0	0	0
Hyperthermia	0	0	0	0	6§	6§	6§
Lethality (24 hours)	0	0	0	0	0	0	0

*Results in this table are measurements made within 30 minutes after intravenous injections. Except where indicated, results obtained either 15 minutes after or 60 minutes after injections were similar. Incidence was considered positive when the indicated measurement was assigned a score of 2 or greater according to the rating system of Irwin (5).

†Characterized by high pelvic position and high stepping.

‡Exophthalmos seen after SCH 32615 at 1000 mg/kg was slight, that seen after morphine at 0.3 and 1 mg/kg was moderate.

§When handled, rats appeared to be warm to the touch.

cantly increase tail-flick latencies (see Fig. 2) is inactive in animals pretreated with naloxone. This demonstrates that SCH 32615's effects are occurring via the expected mechanism.

Acute Side Effect Potential

The acute central nervous system (CNS) side effect potential of SCH 32615 after IV injection was tested using the method of Irwin (5). This test is a series of observations and manipulations designed to evaluate the behavioral, neurological and autonomic side effects of a novel agent. In these experiments, SCH 32615 was tested alone in mice, rats, and squirrel monkeys. Additionally, for comparison, the effects of morphine were also determined in rats and squirrel monkeys.

In mice (Table 2), SCH 32615 had no significant effects on behavior or neurologic function up to 100 mg/kg. At 300 mg/kg, all the animals showed convulsions immediately after the injection. These convulsions lasted for roughly one minute. Following this, the mice appeared normal, except for one animal who showed a decreased transfer reactivity. Importantly, none of the animals died despite the convulsions. This same syndrome was also observed in the 300 mg/kg dose group of animals used in the mouse acetic acid-induced writhing test described above. However, in that study, only 50% of the animals convulsed, and by the time of testing (15 min) all the animals were alive and appeared normal.

In rats (Table 3), SCH 32615 had no significant effects on behavior or neurologic function at doses of 100 and 300 mg/kg IV. At 1000 mg/kg IV, SCH 32615 caused mild sedative effects, including decreases in passivity, ataxia and exophthalmos. At this dose, SCH 32615 also produced some increases in vocalization and transfer reactivity (i.e., the responsivity of the animal to being picked up and moved), the meaning of which is unclear. In contrast, morphine (0.3 mg/kg) affected muscle tone, ataxia, ear twitches, exophthalmos and hyperthermia. At 1 mg/kg of morphine, similar signs were observed.

In squirrel monkeys (Table 4), SCH 32615 (up to 300 mg/kg) had no significant effects. However, morphine produced clear effects, which were particularly notable in terms of the animals' response to the investigator. In this case, the animals were significantly indifferent and passive, and showed decreases in alertness and spontaneous activity. Further, the animals all showed ptosis. Higher doses of morphine could not be tested because of profound respiratory depression, as noted above.

DISCUSSION

In vitro, SCH 32615 is a selective inhibitor of Enk'ase (1). It has not previously been investigated for its in vivo effects. These studies presented the analgesic and potential central side effect risk of SCH 32615 in mice, rats and squirrel monkeys after IV injection. The results show that SCH 32615 produced opiate-like

TABLE 4

EFFECTS OF VEHICLE, SCH 32615 OR MORPHINE ON BEHAVIORAL, NEUROLOGIC AND AUTONOMIC FUNCTION IN SQUIRREL MONKEYS

	Number of 3 Treated Monkeys Manifesting the Indicating Measure at the Indicated Dose (mg/kg IV)				
		SCH 32615		Morphine	
Measure	Vehicle	100	300	0.3	1
Investigator Away from Cage					
Alertness	2	2	1	0	3
Withdrawal	0	0	0	0	0
Vocalization	0	0	0	0	0
Body Position	2	2	1	0	3
Motor Activity	2	2	1	0	3
Investigator Near Cage					
Alertness	0	0	0	0	3*
Indifference	0	0	0	0	2
Vocalization	0	0	0	0	0
Body Position	0	0	0	0	0
Fearfulness	0	0	0	0	1
Motor Activity	0	0	0	0	2*
Investigatory Interacts With Monkey					
Inc. Passivity	0	0	0	0	2*
Aggression	0	0	0	0	0
Autonomic Signs					
Ptosis	0	0	0	0	3*
Piloerection	0	0	0	0	0
Emesis	0	0	0	0	0
Neurologic Signs					
Tremors	0	0	0	0	0
Convulsions	0	0	0	0	0
Catalepsy	0	0	0	0	0
Ataxia	0	0	0	0	0
Lethalities	0	0	0	0	0

*Significantly different than control, based on the criterion of >50% of the treated animals showing a score of ≥2 compared to vehicle based on the scale devised by Irwin (5).

antinociception without producing significantly limiting side effects.

The mouse acetic acid-induced writhing test is one of the most commonly used methods for determining the clinical analgesic potential of a novel agent. In this test, intravenously injected SCH 32615 produced a dose-related decrease in the number of writhes; this action was identical to that seen for morphine. Further, the dose at which significant side effects were seen (i.e., 300 mg/kg) was 100 times the MED for producing analgesic effects. These side effects were severe, insofar as the occurrence of clonic convulsions is always considered severe. However, the effect was very short-lived (i.e., it dissipated within one minute of injection) and was not associated with any lethalities. Additionally, it was not observed in any other species. Therefore, it does not appear as if SCH 32615 is at exceptional risk for producing this effect.

The rat low-yeast inflamed-paw test is a novel test designed to identify the antinociceptive actions of Enk'ase inhibitors (1). In this test, SCH 32615 showed dose-related analgesia. Further, SCH 32615 had similar effects to morphine, insofar as it significantly affected the noninflamed-paw response latencies. This implies that SCH 32615 may be effective against the kinds of pain that morphine can relieve. It was of interest to note that SCH 32615 had no significant side effects at doses up to 100 times those producing analgesia.

Prior to these studies, naloxone-reversible analgesia after the administration of an Enk'ase inhibitor to a nonhuman primate has not been observed. These studies used a modified tail-flick procedure and were able to demonstrate significant increases in response latencies following SCH 32615. This effect was comparable in intensity to morphine, and like standard opioids, the actions of SCH 32615 were antagonized by prior treatment with the narcotic-blocking drug naloxone. Of particular interest was the lack of side effects seen following SCH 32615. This was in marked contrast to morphine which produced severe respiratory depression at doses which were maximally analgesic. Indeed, the effect was so great as to require rescue doses of naloxone to prevent overdosing the animal. Thus at doses producing equal and maximal amounts of analgesia, morphine caused severe side effects while

there were no side effects associated with the injection of SCH 32615.

It is of interest to note the marked difference in sensitivity of the three species to SCH 32615, i.e., the drug was more potent in rodents than in primates. This difference could have been due to the analgesic measure used because morphine showed similar differences. However, an alternative possibility is that the enkephalinergic system in rodents has a more rapid turnover than in primates. Thus an inhibitor of catabolism has a greater effect in a system where more transmitter is released than in a less activated system. Unfortunately, since the rates of enkephalin turnover in monkeys and rodents following noxious stimuli have not been evaluated, it is not possible to compare these species. Regardless, these results highlight the difficulties of projecting pharmacologically relevant doses across species.

In summary, these experiments demonstrate that the intravenous administration of the Enk'ase inhibitor SCH 32615 produces naloxone-reversible analgesic effects in mice, rats and monkeys. Further, this effect is selective, insofar as other behaviors are not affected.

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REFERENCES

1. Chipkin, R. E.; Berger, J. G.; Billard, W.; Iorio, L. C.; Chapman, R.; Barnett, A. Pharmacology of SCH 34826, an orally active enkephalinase inhibitor analgesic. J. Pharmacol. Exp. Ther. 245:829–838; 1988.
2. Chipkin, R. E. Inhibitors of enkephalinase: The next generation of analgesics. Drugs Future 11:593–606; 1986.
3. Dykstra, L. A.; Woods, J. H. A tail-withdrawal procedure for assessing analgesic activity in rhesus monkeys. J. Pharmacol. Methods 15: 263–269; 1986.
4. Hendershot, L. C.; Forsaith, S. Antagonism in the frequency of phenylquinone-induced writhing in the mouse by weak analgesics and nonanalgesics. J. Pharmacol. Exp. Ther. 125:237–240; 1959.
5. Irwin, S. Drug Screening and evaluation of news in animals. In:Nodine, J. M.; Seigler, P. E., eds. Animal and clinical pharmacologic techniques in drug evaluation. Chicago: Year Book Medical Publishers, Inc.; 1964:36–54.
6. Koster, R.; Anderson, M.; DeBeer, E. J. Acetic acid for analgesic screening. Fed. Proc. 18:412; 1959.
7. Llorens-Cortes, C.; Gros, C.; Schwartz, J. C. Study of endogenous Tyr-Gly-Gly, a putative enkephalin metabolite, in mouse brain: Validation of a radioimmunoassay, localization and effects of peptidase inhibitors. Eur. J. Pharmacol. 119:183–191; 1985.
8. Roques, B. P.; Fournie-Zaluski, M.-C.; Soroca, E.; Lecomte, J. M.; Malfroy, B. P.; Llorens, C.; Schwartz, J.-C. The enkephalinase inhibitor thiorphan shows antinociceptive activity in mice. Nature 288:286–288; 1980.
9. Zimmermann, M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16:109–110; 1983.