SR146131: A New Potent, Orally Active, and Selective Nonpeptide Cholecystokinin Subtype 1 Receptor Agonist. II: In Vivo Pharmacological Characterization

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Accepted for publication December 23, 1998

ABSTRACT
SR146131 is a potent and selective agonist at cholecystokinin subtype 1 (CCK,) receptors in vitro. The present study evaluates the activity of the compound in vivo. SR146131 completely inhibited gastric and gallbladder emptying in mice (ED50 of 66 and 2.7 \( \mu \)g/kg p.o., respectively). SR146131 dose dependently reduced food intake in fasted rats (from 0.1 mg/kg p.o.), in nonfasted rats in which food intake had been highly stimulated by the administration of neuropeptide Y (1–36) (from 0.3 mg/kg p.o.), in fasted gerbils (from 0.1 mg/kg p.o.), and in marmosets maintained on a restricted diet (from 3 mg/kg p.o.). SR146131 (10 mg/kg p.o.) also increased the number of Fos-positive cells in the hypothalamic paraventricular nucleus of rats. Locomotor activity of mice was reduced by orally administered SR146131 (from 0.3 mg/kg p.o.). When administered intrastriatally, SR146131 elicited contralateral turning behavior in mice. Furthermore, orally administered SR146131 (0.3–10 mg/kg), also reduced the levels of cerebellar cyclic GMP. Finally, SR146131 (0.1 \( \mu \)g/kg to 1 mg/kg, p.o.) significantly and dose dependently antagonized fluphenazine-induced mouth movements in rats. The CCK, antagonist SR27897B prevented all the effects of SR146131. Conversely, SR146131 was unable to elicit any agonist or antagonist effects in a model of CCK2 receptor stimulation in vivo. SR146131 is a very potent and selective nonpeptide CCK1 agonist in vivo. SR146131 is more potent than any other CCK1 agonists reported to date. Because pharmacodynamic studies suggest that SR146131 should have a high absolute bioavailability, it may be a promising drug for the treatment of eating and motor disorders in humans.

Cholecystokinin (CCK) is a peptide that is secreted in response to food ingestion by endocrine cells within the small intestine and is also abundant in the brain (Crawley and Corwin, 1994). CCK exerts its activity via two G protein-coupled receptor subtypes, the CCK1 (or CCKA) and the CCK2 (or CCKB) receptors (Wank, 1998), which have been cloned from several species. In rodents, the CCK1 receptor is located mainly in the periphery, particularly in pancreatic acinar cells, gallbladder, and ileal muscle (Crawley and Corwin, 1994), but is also present in discrete regions of the central nervous system (Crawley and Corwin, 1994).

In the periphery, via the CCK1 receptor, CCK delays gastric emptying, modulates intestinal motility, stimulates gallbladder contraction, increases bile secretion, and controls pancreatic secretion (Crawley and Corwin, 1994). CCK also plays a key role in satiety: CCK plasma concentrations, which depend on the composition of meals, are increased after meals. CCK decreases food intake in several species including humans (Gibbs et al., 1973; Kissileff et al., 1981; Lieverse et al., 1994), and has been proposed to act as a satiety signal (Gibbs et al., 1973) via CCK1 receptor activation (Dourish et al., 1989). The Otsuka Long Evans Tokushima Fatty (OLETF) rat, which lacks the CCK1 receptor, is insensitive to the anorexigenic action of CCK (Moran et al., 1998). CCK1 receptor antagonists increase food intake in several species (Hewson et al., 1988; Moran et al., 1992), whereas CCK1 agonists have the opposite effect (Asin et al., 1992a,b; Elliott et al., 1994). These and other results suggest that the CCK1 receptor regulates food intake by increasing satiety signals (Jerzy et al., 1990; Reidelberger, 1992), and that CCK1 agonists can be used to reduce food intake.

Experimental evidence also suggests the involvement of CCK in a number of processes in the central nervous system (Crawley and Corwin, 1994). In particular, the CCK1 receptor has been implicated in the control of motor function.

ABBREVIATIONS: CCK, cholecystokinin; CCK8S, sulfated cholecystokinin octapeptide; NPY, neuropeptide Y.
CCK₁ agonists induce turning behavior when injected into the striatum (Worms et al., 1986; Poncelet et al., 1993) and induce hypomotility after systemic administration in rodents (Soar et al., 1989). CCK₁ receptors have also been implicated in the control of cerebellar cyclic GMP (cGMP) levels (Poncelet et al., 1993), a neurochemical parameter shown to vary with locomotor activity (Triner et al., 1981).

CCK also inhibits neuroleptic-induced tardive dyskinesia in animals (Stoessl et al., 1989), an effect reversed by CCK₁ antagonists (Van Kampen et al., 1996), and the CCK analog ceruletid improves some cases of tardive dyskinesia and chorea in humans (Hashimoto and Yanagisawa, 1990). This suggests that CCK₁ receptor stimulation may be beneficial in some neurological disorders.

These observations on the effects of CCK have encouraged the search for CCK₁ agonists that could be of potential therapeutic use, in particular for the treatment of eating disorders and obesity. Peptide analogs of CCK were first synthesized and led to the preparation of compounds with selective CCK₁ agonist activity capable of reducing food intake in a range of species (Asin et al., 1992 a,b; Holladay and Lin, 1992; Simmons et al., 1998). However, these peptide derivatives had very limited activity after oral administration. Nonpeptide compounds that might possess a higher degree of oral bioavailability have also been described. Some 1,5 benzodiazepine derivatives have been found to be equipotent to CCK as anorectic agents following oral administration (Aquino et al., 1996; Hirst et al., 1996; Henke et al., 1997). However, in contrast to the peptide CCK₁ agonists, these compounds exhibited a decreased affinity for the human CCK₁ receptor.

SR146131 is a new, potent, and selective nonpeptide agonist of both human and rodent CCK₁ receptors (Bignon et al., companion paper). In the present study we characterized the activity of SR146131 in a variety of experimental models involving CCK₁ receptor stimulation in vivo. We focused on the effects of the compound on three main areas: the gastrointestinal system, the regulation of food intake in a number of different species, and the modulation of motor function, in particular of dyskinetic behavior. We also evaluated the effects of SR146131 on tests involving the activation of the CCK₂ receptor to verify its selectivity in vivo. The results confirm that the compound exhibits a high potency and selectivity for the CCK₁ receptor in vivo. Furthermore, SR146131 is very active after oral administration, and is therefore, in contrast to previously described CCK₁ agonists, a better candidate for clinical development.

Materials and Methods

All experimental procedures were approved by the “Comité d’Expérimentation Animale” (Animal Care and Use Committee) of Sanofi Recherche and were carried out in accordance with French legislation.

Drugs. SR146131 (2-[4-(4-chloro-2,5-dimethoxyphenyl)-5-(2-cyclohexyl-ethyl)-thiazol-2-ylcarbamoyl]-5,7-dimethyl-indol-1-yl-1-acetic acid) and SR27897B (1-[2-[2]-2-[2]-2-(chloro-phenyl)-2 thiazolyl]-ami nocarboxyl)-indol-acetic acid; Lintritript) were synthesized by Sanofi Recherche. Neuropeptide Y (NPY)(1–36) was obtained from Neosystem (Strasbourg, France). Pentagastrin was from Sigma-Aldrich (Milan, Italy). SR146131 was suspended in Tween 80 and water for oral administration and in DMSO plus water for i.p. injection, except where otherwise stated. SR27897B was suspended in Tween 80 and water for i.p. administration.

Gastric and Gallbladder Emptying in Mice. Female OF1 mice (Iffa Credo, France, 7–10 in each group) weighing 20 to 26 g were fasted for 18 h before the experiments.

For dose-response studies on gastric emptying, SR146131 was given 30 min (i.v.) or 1 h (p.o.) before a charcoal meal (0.3 ml/mouse of 10% charcoal powder, 5% gum arabic, and 1% carboxymethylcellulose suspension in distilled water) was administered. The mice were sacrificed by cervical dislocation 5 min later. The cardia and the pylorus were ligatured, the stomach removed, and the volume of the gastric content was estimated by the weight of the stomach (Gully et al., 1993). SR27897B (3–1000 μg/kg) or its vehicle were administered i.p. 15 min before SR146131.

For time-course studies, SR146131 was given from 15 min to 7 h by i.v. and oral routes before the charcoal meal was administered. Results were expressed as the mean (±S.E.M.) of the weight of the stomach (grams per kilogram of mouse body weight) for each dose group. Statistical differences between drug- and vehicle-treated groups were performed by Student’s t test (when two groups were compared) or by ANOVA followed by Dunnett’s test (for multiple comparisons).

For gallbladder emptying, the mice were sacrificed by cervical dislocation 4 h after SR146131 administration, and the gallbladder was ligatured, removed, and weighed (Gully et al., 1993). Results were expressed as the mean (±S.E.M.) of the weight of the gallbladder (gram per kilogram of mouse body weight) for each dose group. Statistical differences between groups were analyzed using the Kruskall-Wallia test (heterogeneous variance) followed by the Mann-Whitney test with the Holm correction (multiple comparisons).

ED₅₀ and ID₅₀ values were estimated with their 95% CI, using the nonlinear logistic model adjusted with the Levenberg-Marquardt algorithm.

Food Intake in Fasted Rats. Over a period of 10 days before the experiment, male Sprague-Dawley rats (200–230 g, Charles River France, 9–14 per group) were fasted for 18 h, and allowed access to food for only 6 h between 10 AM and 4 PM each day. Water was available ad libitum. At the end of this adaptation phase, rats were administered SR146131 (0.03–3 mg/kg p.o.). One hour after SR146131 administration, a weighed amount of food (A04 standard rat laboratory diet; UAR, France) was introduced into the cage, and food intake was measured (taking into account spillage) 1, 3, 6, and 23 h after SR146131 administration. SR27897B (0.1 mg/kg) or its vehicle were administered i.p. 15 min before SR146131. Results are expressed as the mean ± S.E.M. for each treatment group. A dose × time ANOVA (with repeated measures on the time factor) was conducted on cumulative intakes across time following SR146131 administration. At each time point, post hoc comparisons versus the control group were performed using Dunnett’s test. The determination of the ED₅₀ was made using the four-parameter logistic model according to Ratkovsky and Reedy (1986). The adjustment was obtained by nonlinear regression using the Levenberg-Marquardt algorithm in RS/1 software (BBN Software Product Corporation, Cambridge, MA).

Effect on NPY-Induced Feeding in Nonfasted Rats. Rats were treated with different doses of SR146131 (0.3, 1, and 3 mg/kg p.o.) or vehicle 2 h before an i.v. administration of NPY(1–36) or its vehicle. The peptide was solubilized in 0.9% NaCl with 1% BSA. The injection of NPY(1–36) (2.5 μg/μl) or vehicle was made free-hand into the lateral ventricle (i.e.v.) of nonrestrained rats by means of a 50-μl microsyringe with a 10-mm calibrated needle (final length below the skin: 3.5 mm). A known amount of food (A04 standard rat laboratory diet; UAR) was immediately introduced into the cage, and food intake was measured (taking into account spillage) over the following 1, 2, 3, and 4 h. Statistical comparisons were performed using a dose × time ANOVA, followed by Dunnett’s test, as described above.
Food Intake in Fasted Gerbils. Gerbils (60–80 g, CERJ, France) were fasted for 24 h before the test. On the day of test, groups of 10 gerbils were given SR146131 (0.1 or 1 mg/kg p.o.) or its vehicle and immediately placed individually in translucent boxes (8 × 11 × 24 cm) with a weighed amount of their regular food (A04 standard laboratory diet; UAR). The amount eaten by each gerbil was measured by weighing food (including spillage) before and then 1 h and 2 h after treatment. Statistical analysis was performed using ANOVA followed by Dunnett’s test.

Food Intake in Marmosets. Marmosets (Callithrix jacchus) were obtained from Sanofi Winthrop Ltd., Alnwick (UK). All the animals were domestically bred. The marmosets were housed in a room maintained at a temperature of 24 ± 1°C and air conditioned to prevent overheating. The relative humidity was maintained at 50 to 60% by steam injection, and forced ventilation produced 12 air changes per hour, the incoming air being filtered through 5-μm filters. Timed switching provided fluorescent lighting for 12 h per day from 7 AM to 7 PM. This is preceded and followed by a short period of twilight, but a dim lighting was maintained throughout the night.

Twelve male and 11 female adults, 350 to 500 g (3–5 years old), were housed individually or in single sex pairs, in a total of 17 cages. The cages (North Kent Plastic Cages Ltd., Dartford, Kent, UK) measured 76 cm high, 57 cm wide, and 61 cm deep, with a wire mesh front and floor, three wooden perches and an open-ended nesting shelf. Softwood shavings were provided as absorbent bedding in all cages. The animals were fed with a standard primate pellet diet (Mazzuri Primate; SDS, Vigny, France), and supplements of fresh fruits, boiled eggs, and vitamins disposed on a plate. During the week before the first treatment, and during the experimental session, food was available to the animals for 3 h only in the afternoon (water was available ad libitum).

SR146131 was administered p.o. by gavage at the doses of 0.3, 1, 3, and 10 mg/kg in a volume of 1 ml/kg. Animals were treated acutely, twice a week (Tuesday and Thursday) with either SR146131 or its vehicle, according to a crossover design so that the effects of all treatments were evaluated on each animal. The animals were fasted overnight (from 20 h) before each experimental day. One hour after treatment, a preweighed amount of food was introduced into the cage for the following 3 h. After this time, residues were removed and spillage collected, and the animals’ food intake was measured. For the cages containing two animals, the mean food intake was used. Differences between food intake after vehicle or each dose of SR146131 were assessed by Dunnett’s test after ANOVA with repeated measures using RS/1 software (BBN Software Product Corporation).

Expression of Fos in Paraventricular Nucleus of Rats. Male Sprague-Dawley rats (250–300 g, Charles River, France) received oral administrations of SR146131 (3.0 or 10 mg/kg) or the vehicle, and were sacrificed 2.5 h later as described below for the immunohistochemical detection of Fos. To examine the effects of the CCK₁ receptor blockade on SR146131-induced Fos-like immunoreactivity, SR27897B (0.3 mg/kg) was injected i.p., 30 min before the oral administration of SR146131 (10 mg/kg) and rats were sacrificed 2.5 h after SR146131 treatment.

Rats were anesthetized with sodium pentobarbital (80 mg/kg i.p.) and perfused transcardially with saline followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Brains were removed and allowed to postfix overnight in 4% paraformaldehyde. Fifty-micrometer coronal sections were cut from each brain using a vibratome. Immunohistochemistry was performed on free-floating tissue sections according to a standard avidin-biotin-peroxidase procedure using an anti-Fos sheep polyclonal antibody (Genosys Biotechnologies Inc., Cambridge, UK, CRB OA-11–824) directed against residues 2 to 16 of the N-terminal region of the Fos protein. Sections were rinsed with 0.02 M PBS and then pretreated for 10 min at room temperature with the same buffer containing 0.3% hydrogen peroxide. Next, they were rinsed in PBS three times, incubated with PBS containing 5% normal rabbit serum (Vector Laboratories, Burlingame, CA) for 1 h at room temperature, and placed for 72 h at 4°C in the Fos primary antiserum (diluted 1:8000) solution in PBS containing 1% normal rabbit serum and 0.3% Triton X-100. Thereafter, the sections were incubated successively with a biotinylated rabbit anti-sheep secondary antibody (Vector Laboratories; diluted 1:300) for 2 h at 37°C and with avidin-biotinylated horseradish peroxidase complex (Vector Laboratories; diluted 1: 200) for 3 h at room temperature in PBS containing 0.1% Triton X-100. The reaction product was visualized with diaminobenzidine in the presence of nickel. Sections were mounted, air dried, dehydrated, and covered with acryl. Omission of the primary antibody from the immunohistochemical procedure or preadsorption of this antibody with a synthetic peptide corresponding to the N-terminal antigenic sequence of the Fos protein (Genosys, OP-11–3210) eliminated Fos-like immunoreactivity.

Sections processes for chromogenic detection of Fos-like immunoreactivity were imaged through a Leica DMRB microscope and the Fos-like immunoreactive signal was quantified with an image analysis system (Samba, Uniofg; Grenoble, France) by counting the number of Fos-positive cells within a 1 mm² area located at the level of the hypothalamic paraventricular nucleus (panel 25 of the atlas of Paxinos and Watson, 1986). Only visual objects that met the appropriate size and optical density criteria (cells showing gray levels between 0 and 160/170, total range 0–255), were automatically counted as Fos-immunoreactive cells. For each animal, three serial sections were counted bilaterally and the data averaged. Data from treatment groups, presented as the mean ± S.E.M. number of Fos-immunoreactive cells, were submitted to one-way ANOVA followed by Dunnett’s or Newman-Keuls’ tests, as appropriate.

Locomotor Activity in Mice. Male Swiss CD1 mice (Charles River, France) weighing 19 to 29 g were used. Animals had free access to food (A04 standard laboratory diet; UAR) and water. Testing of locomotor activity was carried out in individual Perspex boxes (24 × 13 × 12 cm). The boxes were fitted with four pairs of infrared photocells, two pairs positioned in the front, and two in the back of the box. Activity was measured as the number of photocell interrruptions. The locomotor cages were connected via a laboratory interface (Imetronic, Pessac, France) to a microcomputer system that recorded photocells count. SR146131 was administered p.o. at doses of 0.3 to 10 mg/kg. SR27897B was administered i.p. at the dose of 0.1 mg/kg. The compounds were prepared extemporaneously and administered in a volume of 10 ml/kg to groups of 10 mice. In each independent experiment, control mice received the corresponding vehicle.

Mice were placed singly in an activity cage 60 min after treatment with SR146131. SR27897B was administered 30 min before SR146131. Motility was assessed over a period of 30 min for SR146131. Results were expressed as the mean (±S.E.M.) of the locomotor scores for each dose group. Differences between control and treated groups were assessed by ANOVA followed by Dunnett’s test. The ED₅₀ was determined using the four-parameter logistic model of Ratkovsky and Reedy (1986). The adjustment was obtained by nonlinear regression using the Levenberg-Marquardt algorithm in RS/1 software (BBN Software Product Corporation).

Turning Behavior in Mice. SR146131 (0.01–1 pg) was solubilized in DMSO (1 mg/ml), diluted to the required concentrations with water, and injected (in 1 μl) into one striatum in awake, hand-restrained female CD1 mice (25–30 g; Charles River, France). After injection, the animals were placed individually in Plexiglas cages (10 × 10 × 15 cm). The number of complete contralateral rotations (away from the injection site) and ipsilateral rotations (toward the injection site) were visually recorded and cumulated over three 2-min periods (2–4, 5–7, and 8–10 min post injection), and the mean (±S.E.M.) number of turns in 6 min was calculated. SR27897B was administered i.p. 30 min before intrastratal injection of SR146131. Differences between control and treated groups were assessed by ANOVA, followed by Dunnett’s test.
Tardive Dyskinesia in Rats. Two-month-old male Sprague-Dawley rats (Charles River, France) were treated with fluphenazine decanoate (25 mg/kg; Sanofi Winthrop) administered i.m. into the hindlimb once every 3 to 4 weeks until the test sessions. In test sessions, animals were allowed to habituate for at least 60 min in Perspex boxes (23 × 13 × 13 cm). The duration of mouth movements including vacuous nondirected chewing, tremor of the jaws, grinding of the teeth, and tongue protrusions were continuously recorded for 15 min (Stoessel et al., 1989). Values are the mean (±S.E.M.) duration of abnormal movements during the 15-min observation period. In the dose-response study, SR146131 was administered p.o. 60 min before the test period, 47 weeks following the initial injection of fluphenazine to the rats. In the antagonism study, SR146131 was administered p.o. and SR27897B i.p., 60 and 45 min, respectively, before the test period, 67 weeks after the initial injection of fluphenazine. Statistical analysis was performed using ANOVA, followed by Dunnett’s or Newman-Keuls’ tests, as appropriate.

Determination of Cerebellar cGMP in Mice. Experiments were performed in the morning, and special care was taken to avoid stressful manipulation. Male Swiss mice (18–20 g) were pretreated with SR146131 (0.1–10 mg/kg, p.o.) or its vehicle 60 min before sacrifice by focused microwave irradiation (3.4 kW per cm 2/1.6 s; Sarcron 8000, Sairem, Villeurbanne, France). The cerebellar cortex was dissected and homogenized with a polytron in 1.75 ml of ethanol. The homogenates were centrifuged at 1500g at 4°C, and the supernatants were stored at −4°C until radioimmunoassay using kits with succinyl-tyrosine [ 125I]methyl ester derivative of cGMP radiolabeled antigen (NEL, Boston, MA). Protein contents were measured by the method of Bradford (1976). SR27897B was injected i.p. at various doses 30 min after SR146131 administration. The results are expressed as a percentage of the control response. The effect of SR146131 at various doses versus the control group, and the effect of SR146131 + SR27897B versus SR146131 alone were tested using ANOVA followed by Dunnett’s or Newman-Keuls’ tests, as appropriate.

Gastric Acid Secretion in Anesthetized Rats. Gastric secretion in anesthetized rats was measured by the stomach-lumen perfusion technique of Ghosh and Schild (1958) with minor modifications. Female Crl:CDBR rats (Charles River, Italy), weighing 220 ± 20 g were used. Rats were fasted for 24 h, then anesthetized with ethyl-urethane (1.2 g/kg, i.p.) and, after laparatomy, the stomach was cannulated at the cardiac and pyloric ends and perfused with warm saline (NaCl 154 mM, 37°C, pH 5.5) at a rate of 1 ml/min, using a peristaltic pump. Fractions of 5 ml were automatically collected and the pH of each fraction was determined (Amel 338 pH meter) and recorded by an automatic apparatus on which samples from eight rats could be measured simultaneously. The pH values were transformed into microequivalents of H+ secreted in the 5-min fraction.

After determination of basal pH (six 5-min fractions of spontaneous gastric secretion), pentagastrin or SR146131 were given i.v. (dissolved in polyethylene glycol 400/glycerol/water, 22/1.2/15), and modifications of the pH value (eight fractions of 5-min gastric secretion) were recorded. To assess the inhibitory effect of SR146131 on pentagastrin secretion, the antagonist was also given i.v. 5 min before a submaximal dose of pentagastrin (4 μg/kg, i.v.). The percentage of stimulation of basal acid secretion by pentagastrin was evaluated by the equation:

\[ \% \text{ stimulation} = 100 \left( 1 - \frac{\mu \text{Eq H}^-/\text{basal} \mu \text{Eq H}^-}{} \right) \]

The effect of SR146131 on a submaximal dose of pentagastrin (4 μg/kg, i.v.) was calculated as percentage of inhibition of pentagastrin effect. The results are expressed as mean ± S.E.M. Statistical analysis of the data was performed by paired Student’s t test (when two groups were compared) or by paired one-way ANOVA followed by Dunnett’s test (for multiple comparisons).

Results

Gastric and Gallbladder Emptying in Mice. SR146131 completely inhibited gastric emptying in mice at a very low dose after oral administration (Fig. 1), with an ED50 of 66 μg/kg (48–87, 95% CL). The efficiency of SR146131 by this route was comparable with that obtained after i.v. administration [ED50 of 38 μg/kg (29–72, 95% CL)].

Time-course studies (Fig. 2) showed that 100 μg/kg SR146131 led to a significant inhibition of gastric emptying lasting 3 (t = 2.71, P < .05) and 5 h (t = 4.12, P < .001) after its administration by the oral and i.v. routes, respectively. The increase of the area under the curve (AUC) induced by SR146131 (ΔAUC: AUCSR146131 − AUCvehicle) was almost identical for the two routes of administration. The ratio of the ΔAUC between the i.v. and oral routes was estimated to be around 70%, suggesting that SR146131 should have a high absolute bioavailability.

As shown in Fig. 3, the selective CCK1 antagonist SR27897B dose dependently reduced the inhibition of gastric emptying induced by 300 μg/kg p.o. of SR146131 with an ID50 of 12.6 μg/kg i.p. (6.9–20.4, 95% CL). SR146131 decreased gallbladder volume in mice with a high efficiency after oral administration (Fig. 4), with an ED50 of 2.7 μg/kg (1.2–7.1, 95% CL). However, the maximum effect of SR146131 on the gallbladder occurred at 3 to 5 h after drug administration (data not shown), whereas its effect on gastric emptying occurred more rapidly (0.5–1 h).
As with gastric emptying, the selective CCK₁ antagonist SR27897B dose dependently antagonized the inhibition of gallbladder emptying induced by 100 μg/kg p.o. of SR146131, with an ID₅₀ of 12.5 μg/kg i.p. (9.1–18.7, 95% CL) (Fig. 4B).

Food Intake in Rats. SR146131 induced a dose-related decrease in food intake in fasted rats (Fig. 5). This effect was highly significant from the dose 0.1 mg/kg p.o. on the cumulative 3-h food intake, with an ED₅₀ of 0.43 mg/kg (0.29–0.67, 95% CL). Food intake over the 23-h period was significantly decreased by 42% and 63% at 1 and 3 mg/kg SR146131, respectively. SR27897B (0.1 mg/kg i.p.) totally prevented the
hypophagic activity induced by 1 mg/kg of SR146131 (results not shown).

In nonfasted rats, NPY(1–36) induced a potent stimulation of food intake. This effect was highly significant during the first hour of feeding, and was maintained over the entire 4-h period studied. SR146131 induced a marked, dose-related decrease in the food intake stimulated by the administration of NPY(1–36). This effect was significant from the dose of 0.3 mg/kg p.o. on the 2-h food intake, and was sustained over the 4 h during which food intake was measured (Fig. 6).

**Food Intake in Gerbils.** Oral administration of SR146131 to gerbils reduced the food intake of these animals. At 1 h after treatment, there was a significant reduction (33%), of food intake at the dose of 1 mg/kg SR146131, but the effect of the dose of 0.1 mg/kg did not attain statistical significance at this time point. At 2 h after treatment, however, the reduction of food intake produced by both doses of SR146131 was statistically significant (33% and 45%, respectively).

**Food Intake in Marmosets.** Oral administration of SR146131 to marmosets significantly and dose dependently reduced the food intake of these animals (Table 1). The first active dose was 3 mg/kg p.o., which reduced the 3-h food intake by 23% \((t = 4.28, P < .05)\), and this effect was somewhat greater at the dose of 10 mg/kg p.o. (28% reduction, \(t = 5.26, P < .05)\).

**Expression of Fos in Paraventricular Nucleus of Rats.** Oral administration of SR146131 at the doses of 3 and 10 mg/kg (Fig. 7) increased the number of Fos-positive cells in the hypothalamic paraventricular nucleus as compared with vehicle-treated rats \([n = 5]\); SR146131 (3 mg/kg): 48 ± 7 (\(n = 5\)); SR146131 (10 mg/kg): 181 ± 18 (\(n = 7\)), while rats receiving SR27897B (0.3 mg/kg i.p.) before oral administration of SR146131 (10 mg/kg, Fig. 7) had Fos counts comparable with those of vehicle-treated rats \([n = 5]\); SR27897B + SR146131: 51 ± 12 (\(n = 4\)). SR27897B (0.3 mg/kg) administered alone had no effect on Fos expression (not shown). ANOVA yielded a significant main effect for drug treatment \([F(3,20) = 20.27, P < .01]\) and Dunnett's test showed that only animals treated with 10 mg/kg of SR146131 differed significantly \((p < .01)\) from vehicle-treated rats with respect to the number of Fos-immunoreactive cells. Post hoc Newman-Keuls test indicated that the pretreatment with SR27897B significantly prevented \((p < .01)\) the Fos-inducing effect of SR146131 (10 mg/kg).

**Locomotor Activity in Mice.** SR146131 induced a dose-related decrease in locomotor activity (Table 2). This effect was highly significant at the doses of 0.3 to 10 mg/kg p.o., with an ED\(_{50}\) of 0.7 mg/kg \((0.2–1.4, 95\%\ CL)\). SR27897B at 0.1 mg/kg i.p. totally antagonized the suppression of locomotor activity induced by 3 mg/kg p.o. of SR146131 (data not shown).

**Turning in Mice.** The unilateral striatal application of SR146131 at 0.01, 0.03, 0.1, and 1 pg produced contralateral circling behavior with a significant and dose-dependent effect (Fig. 8A). This was associated with a slight but significant reduction of ipsilateral turns. In light of these results, the antagonistic activity of SR27897B was studied on the turning produced by 0.1 pg of SR146131. Figure 8B shows that SR27897B dose dependently and significantly counteracted the turning induced by SR146131, with an ID\(_{50}\) of 0.1 mg/kg i.p. \((0.038–6.6, 95\%\ CL)\), ipsilateral turns were restored only at the highest dose of SR27897B.

**Tardive Dyskinesia in Rats.** Figure 9A shows that SR146131 (0.1 μg/kg to 1 mg/kg, p.o.) significantly and dose dependently antagonized fluphenazine-induced mouth movements with an ID\(_{50}\) of 12 μg/kg \((0.6–386.6, 95\%\ CL)\). Figure 9B shows that SR146131 (1 mg/kg p.o.) significantly antagonized \((58\%)\) the tardive dyskinesia. SR27897B (0.3 mg/kg i.p.) did not significantly modify \((26\%)\) the movements induced by fluphenazine, but significantly counteracted the antagonism induced by SR146131.

**cGMP Levels in Cerebellar Cortex of Mice.** Oral administration of SR146131 produced a dose-dependent decrease of cGMP levels in the cerebellum in mice, from the dose of 1 mg/kg p.o. (Fig. 10), with an apparent maximum effect of about 40% reduction. The effect of 10 mg/kg p.o. SR146131 on cGMP levels was abolished by the administration of 3 μg/kg i.p. SR27897B (Fig. 10).

**Gastric Acid Secretion in Anesthetized Rats.** Pentagastrin (4 μg/kg i.v.) increased gastric acid secretion \([t(3) = 8.02, P < .01]\) from control values of 1.3 ± 0.09 μEq H\(^+\) to 4.06 ± 0.36 μEq. In contrast, SR146131 (0.3 or 1 mg/kg i.v.) did not modify the secretion of gastric acid \([t(4) = 0.74, P > .01]\).
.05 and $t(2) = 1.59, P > .05$, respectively). At these same doses, SR146131 did not inhibit the increase $[F(2,6) = 1.43, P > .05]$ of gastric acid secretion induced by pentagastrin (results not shown). Thus, SR146131 neither stimulated nor inhibited the CCK$_2$ receptor in vivo.

**Discussion**

The accompanying paper (Bignon et al., 1999) on SR146131 describes the compound’s effects as a potent and selective agonist at both human and rodent CCK$_1$ receptors in vitro. The present paper evaluated the drug’s effects in vivo, and shows clearly that SR146131 can mimic, in a variety of test systems and in several species, a wide range of the effects of sulfated cholecystokinin octapeptide (CCK8S), which have previously been attributed to the stimulation of CCK$_1$ receptors but not those related to the stimulation of CCK$_2$ receptors.

SR146131 inhibited gastric emptying in mice, and also decreased gallbladder volume in this species after administration of low oral doses. SR146131 also reduced food intake

![Fig. 7. Photomicrographs of Fos-like immunoreactivity in paraventricular nucleus of rats treated with vehicle (A), 10 mg/kg p.o. SR146131 (B), or pretreated with SR27897B (0.3 mg/kg, i.p.) 30 min before oral administration of SR146131 (C). Scale bar, 350 μm.](image)

![Fig. 8. Effect of SR146131 on turning behavior in mice. A, SR146131 (0.01–1 pg) was injected in 1 μl DMSO into one striatum in awake, unrestrained mice. Number of complete contralateral (away from injection site) and ipsilateral rotations (toward injection site) was visually recorded and cumulated over three 2-min periods. B, SR27897B was administered i.p. 30 min before intrastriatal injection of SR146131. Results are expressed as mean ± S.E.M for each treatment group. ANOVA for contralateral (A) rotations: $F(4,79) = 6.07, P < .01$; Ipsilateral rotations: $F(4,79) = 5.11, P < .01$; contralateral (B) rotations: $F(5,90) = 4.77, P < .01$; ipsilateral rotations: $F(5,90) = 4.26, P < .01$, post hoc tests, *$p < .05$; **$p < .01$, Dunnett’s test versus vehicle group.](image)

**TABLE 2**

<table>
<thead>
<tr>
<th>Effect of SR146131 on locomotor activity in mice</th>
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<tr>
<td>SR146131 Locomotor activity in 30 min</td>
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<tr>
<td>mg/kg p.o.</td>
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<tr>
<td>0</td>
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<tr>
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ANOVA, $F(4,45) = 10.3, P < .01$; post hoc tests, *$P < .01$ Dunnett’s test versus vehicle.
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Fig. 9. Effect of SR146131 on fluphenazine-induced tardive dyskinesia in rats. SR146131 and SR27897B were administered by oral and i.p. routes 60 and 45 min, respectively, before observation period. Duration of mouth movements was continuously recorded for 15 min. Results are expressed as mean ± S.E.M. for each treatment group. A, ANOVA: F(5,59) = 4.1, P < .01 post hoc tests, *p < .05, **p < .01 Dunnett's test versus vehicle; B, ANOVA: F(3,92) = 4.37, P < .01, post hoc tests: Newman-Keuls test, *p < .05 versus the vehicle, #p < .05 versus SR146131.

in two rodent and one primate species. The compound reduced food intake in fasted rats, and in nonfasted rats in which food intake had been highly stimulated by the administration of NPY(1–36). Food intake was also reduced by oral administration of SR146131 in fasted gerbils, and in marmosets maintained on a restricted diet. Furthermore, SR146131 was able to increase the number of Fos-positive cells in the hypothalamic paraventricular nucleus of rats, indicating an activation of the neurons in this nucleus, which is known to be part of the anatomical pathway implicated in the regulation of food intake by CCK via CCK1 receptors (McCann and Rogers, 1991; Mönnikes et al., 1997). In all the experimental models in which the effect of SR27897B was evaluated, the CCK1 receptor antagonist was able to antagonize the effects of SR146131.

SR146131 was also shown to act on a variety of parameters related to locomotor control. When administered intrastrially, SR146131, like CCK-8S (Worms et al., 1986), elicited turning behavior in mice. Locomotor activity of mice was reduced by orally administered SR146131. Furthermore, orally administered SR146131 reduced the levels of cerebellar cGMP, which have previously been shown to vary with locomotor activity (Triner et al., 1981). However, the large dose-dependent changes in locomotor activity were accompa-

nied by modest variations in cGMP levels. This indicates that the relationship between these two phenomena is incomplete as already suggested by differences in the ability of devazepide versus SR27897B to inhibit CCK-induced hypolocomotion versus CCK-induced reduction in cerebellar cGMP levels. (Poncelet et al., 1993). Finally, SR146131 (0.1 μg/kg to 1 mg/kg, p.o.) significantly and dose dependently antagonized mouth movements induced by long-term fluphenazine treatment, considered to be an animal model of neuroleptic-induced tardive dyskinesia (Stoessl et al., 1989). The CCK1 antagonist SR27897B prevented the effects of SR146131, confirming that these effects of the compound, like those of CCK8S in the same model (Van Kampen et al., 1996), were effectively attributable to the stimulation of CCK1 receptors.

In contrast to its efficacy in experimental models implicating the CCK1 receptor, SR146131 was unable to elicit any CCK4-like effects in a model of CCK2 receptor stimulation in vivo (the enhancement of gastric acid secretion in rats). Furthermore, CCK2 agonists have been found to induce signs of panic in humans and anxiety in rodents (van Megen et al., 1996), but we were unable to find (our unpublished data) any anxiogenic effects of SR146131 in the rat or in the marmoset, a species in which the behavioral signs associated with anxiety are well documented (Carey et al., 1992). Finally, SR146131 did not inhibit CCK2 receptor-mediated events in vivo, because it did not inhibit the effect of pentagastrin on gastric secretion. Therefore, these data confirm that SR146131 retains its profile as a potent and selective CCK1 receptor agonist in vivo in a number of animal species.

Very few orally active nonpeptide CCK1 agonists have been described: the 1.5 benzodiazepine derivatives GW7854 (Hirst et al., 1996; Aquino et al., 1996) and the most recently described GW5823 (Henke et al., 1997) bind to human CCK1 receptors with moderate affinity and selectivity over the CCK2 receptor. In mice, by the oral route these compounds
stimulate gallbladder emptying with ED_{50} of 10 to 55 nmol/kg (approximately 5–25 μg/kg), which is comparable with the activity of SR146131 (which has an ED_{50} of 2.7 μg/kg). GW5823 (Henke et al., 1997) and GW7854 were effective in reducing food intake to 40% and 58%, respectively, of vehicle controls when given orally at a dose of 10 μmol/kg (approximately 5 mg/kg). Although methodological differences preclude a direct comparison, SR146131 would seem to be more potent than these molecules for inhibiting food intake in rats, because it significantly inhibited food intake from the dose of 100 μg/kg. These results also suggest that although the ability to stimulate gallbladder emptying is a very sensitive and selective test for the detection of CCK₁ agonists, it cannot be used to predict the potency of the compounds to inhibit feeding, possibly due to the existence of a large quantity of spare receptors on this tissue (Simmons et al., 1998). The potency of SR146131 by the i.v. and oral routes is similar, and the duration of the inhibition of gastric emptying in mice is also comparable by these routes, suggesting that SR146131 should have a high absolute bioavailability. This does not seem to be the case for GW5823 and several close analogs, which suffer from poor oral bioavailability and a relatively high clearance in the rat (Henke et al., 1997).

In view of the potency, selectivity, and oral bioavailability of SR146131, this compound would appear to be one of the first nonpeptide CCK₁ receptor agonists worthy of consideration for clinical development. The therapeutic field with which CCK₁ receptor stimulation has the most frequently been associated has been that of obesity. For many years, evidence has been accumulating on the physiological role of CCK as an important satiety signal (Gibbs et al., 1973; Lee et al., 1994), a concept supported by the observation that CCK has been found to efficiently suppress food intake in humans (Kissileff et al., 1981; Lieverse et al., 1994). Conversely, many studies indicate a role for NPY as a physiological appetite stimulant (Stanley and Leibowitz, 1985; Lee et al., 1994). The ability of SR146131 to reduce food intake in a number of species at low oral doses and to powerfully block the appetite-stimulating effects of NPY suggests that its anorexigenic potential should be evaluated in humans.

Another possible field in which the effects of this compound might be beneficial is the alleviation of the tardive dyskinesia that occurs as a side effect of chronic neuroleptic administration. Although the new generation of atypical antipsychotic agents may help to alleviate concerns about tardive dyskinesia, this disorder remains a significant clinical problem for both patients and physicians, because the older antipsychotic drugs are still very frequently prescribed. Many cases of tardive dyskinesia are mild, but patients with moderate to severe forms of tardive dyskinesia present a great challenge and frequently require medication to suppress their dyskinesias. A variety of suppressive agents have been tried with limited success and no fully satisfactory treatment strategy has as yet emerged (Egan et al., 1997). The present results with SR146131 suggest that the selective stimulation of CCK₁ receptors may provide an effective means of attenuating neuroleptic-induced tardive dyskinesias.

In conclusion, SR146131 is active at low oral doses in a wide variety of experimental models of CCK₁ receptor activation. This pharmacological profile, together with the compound's high potency and selectivity for the human CCK₁ receptor (Bignon et al., companion paper), suggests that SR146131 may be an excellent candidate for the treatment of clinical conditions such as obesity and tardive dyskinesia.

Acknowledgments

We thank Dr Danielle Gully for comments on the manuscript, Josephine Beuste, Roselyne Calassi, MariaGrazia Dozio, Claude Dufour, Michele Fournier, MariaGiovanna Marongiu, Nadine Gonolas, Joasette Guittard, Martine Keane, Massimo Meleri, Christine Menet, Yves Rouzier, and Jean-Jacques Storme for excellent technical support, and Marilyne Labide and Danielle Lasserre for expert secretarial assistance. Christophe Aut and Noëlle Boussac are gratefully acknowledged for assistance with the statistical analysis of the data.

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