ELSEVIER

European Journal of Pharmacology 367 (1999) 315–323

ejp

Effect of deramciclane, a new 5-HT receptor antagonist, on cholecystokinin-induced changes in rat gastrointestinal function

Gábor Varga [a,b,*], Krisztina Kordás [a], Beáta Burghardt [a], István Gacsályi [c], Gábor Szénási [c]

[a] *Institute of Experimental Medicine, Hungarian Academy of Sciences, PO Box 67, H-1450 Budapest, Hungary*
[b] *Department of Oral Biology, Semmelweis University of Medicine, Budapest, Hungary*
[c] *Division of Preclinical Research, EGIS Pharmaceuticals, Budapest, Hungary*

Received 4 November 1998; revised 15 December 1998; accepted 18 December 1998

Abstract

Recent studies suggested that serotonin receptors may be involved in modulating the actions of cholecystokinin (CCK) in the gastrointestinal tract. The present work was designed to compare the effects of deramciclane, a recently developed serotonin-2 ($5\text{-HT}_{2A/2C}$) receptor antagonist, and lorglumide, a CCK_A receptor antagonist, on exogenous and endogenous CCK-induced pancreatic enzyme secretion and pancreatic growth, as well as on the emptying of the stomach and the gallbladder. Pancreatic secretory function was tested while CCK release was evoked by diversion of bile-pancreatic juice in rats. Adaptive growth of the pancreas was induced by chronic intragastric administration of camostate, a potent synthetic trypsin inhibitor in rats. Gastric emptying of a noncaloric test meal was investigated in response to intraduodenal intralipid infusion, also in rats. In fasted mice, gallbladder emptying was examined in response to intragastric egg yolk administration. In rats, diversion of bile-pancreatic juice from the duodenum stimulated pancreatic amylase secretion. This action was blocked by deramciclane and by lorglumide. Pancreatic hypertrophy and hyperplasia induced by chronic camostate administration was also suppressed by both the serotonin- and the CCK-receptor antagonists. Intraduodenal administration of intralipid induced a significant delay in gastric emptying. This effect was inhibited by both deramciclane and lorglumide in rats. In mice, intragastric administration of egg yolk elicited an accelerated release of bile from the gallbladder. Prior treatment with either deramciclane or lorglumide abolished this response. Lorglumide was able to inhibit the functional responses elicited by exogenous CCK administration in both pancreas, stomach and gallbladder, while deramciclane was not effective under such circumstances. Our data show that deramciclane inhibited the effects of CCK on pancreatic, gastric and gallbladder function when its endogenous release was stimulated, but did not alter the effects of exogenously administered peptide. These results suggest that serotonin, primarily via 5-HT_{2A} receptors, may modulate CCK-mediated gastrointestinal functions in rats.

Keywords: 5-HT receptor; 5-HT (5-hydroxytryptamine, serotonin); CCK (cholecystokinin); Pancreas; Gastric emptying; Gallbladder

1. Introduction

It is well known that cholecystokinin (CCK) belongs to the group of brain-gut peptides: it functions both as a neuropeptide and as a gut hormone (Dockray, 1989). In the gut, its primary roles are thought to be the physiological regulation of pancreatic enzyme secretion and gallbladder contraction (Chey, 1993). In vivo experiments have shown that both endogenous and exogenous CCK increases enzyme output from the pancreas and stimulates gallbladder emptying in different animal species, including humans (Liddle, 1994). The presence of specific receptors for CCK-like peptides in the mammalian pancreas and gallbladder was confirmed in in vitro studies with pancreatic acini and gallbladder strips, respectively (Liddle, 1994). Along with its effect on secretion, the ability of CCK alone, or in combination with secretin, to produce hyperplasia and hypertrophy of the exocrine pancreas has been well documented (Solomon, 1981; Fölsch, 1984).

Although the pancreas and the gallbladder are the principal target organs of CCK in the gastrointestinal tract, CCK receptors present throughout the gut (Woodruff and Hughes, 1991), including smooth muscle of the stomach (Miller, 1984). It is, therefore, conceivable that CCK has a physiological role not only in the stimulation of pancreatic

* Corresponding author. Tel.: +36-1-210-0819; Fax: +36-1-210-0813; E-mail: varga-g@koki.hu

0014-2999/99/$ - see front matter
PII: S0014-2999(98)00958-3

secretion and gallbladder contraction, but also in the regulation of gastrointestinal motility. From a physiological point of view, CCK is probably one of the peptides involved in the regulation of peristalsis (Mayer, 1994). Gastric emptying is one of the most important motor functions in the gut. It limits the rate of absorption of nutrients and drugs by controlling delivery into the small intestine. The rate of delivery is modulated by feedback from nutrients in the small intestine, and by the central nervous system via the vagus and sympathetic nerves and the release of a variety of hormones (Mayer, 1994). CCK delays emptying of the gastric contents in both animals and humans (Mayer, 1994). In rats, both exogenous and endogenous CCK delays, while CCK_A receptor antagonists accelerate gastric emptying (Green et al., 1988; Scarpignato et al., 1993; Varga and Scarpignato, 1996).

Recent studies have suggested that 5-HT_3 and 5-HT_2 receptor antagonists are able to inhibit the pancreatic secretory response to endogenous CCK stimulation (Li and Owyang, 1996a; Li et al., 1996b). Furthermore, serotonin and serotonin receptor immunoreactivities have also been demonstrated in the pancreas, stomach and intestine (Bonhaus et al., 1995; Kuemmerle et al., 1995; Kirchgessner et al., 1996; Sha et al., 1996; Berger and Fehér, 1997), but their physiological role is not completely understood. Deramciclane is a novel anxyolitic compound (Gacsályi et al., 1988) that has been reported to have marked affinity for both 5-HT_{2A} and 5-HT_{2C} receptors (Gacsályi et al., 1988, 1996, 1997). It has been clearly shown that deramciclane is a 5-HT_2 receptor antagonist both at central and peripheral locations (Gacsályi et al., 1988, 1996, 1997). In the present investigation, the effect of deramciclane was evaluated on CCK-induced changes of gastrointestinal function in several experimental models in rats and mice. Endogenous CCK release was induced in four different, well-characterised ways: diversion of bile-pancreatic juice from the duodenum (Louie et al., 1986; Varga et al., 1991); intragastric administration of camostate, a potent releaser of endogenous CCK (Göke et al., 1986; Douglas et al., 1990); intraduodenal intralipid infusion (Hölzer et al., 1994); and intragastric egg yolk administration (Makovec et al., 1987a,b; Gully et al., 1993). The functional activity of the pancreas, stomach and gallbladder was measured and the modulatory effect of deramciclane and the CCK_A receptor antagonist lorglumide was compared.

2. Materials and methods

2.1. Pancreatic secretory studies

Male Wistar rats weighing 280–360 g were used. They were purchased from LabTech Kft (Hungarian branch of Charles River Europe) and were used at least one week after their arrival at the laboratory. The animals were housed at constant temperature (24°C) and under a 12 12-h light cycle and were fed on standard rat chow at libitum.

Rats were prepared and maintained as previously described (Varga et al., 1991; Reidelberger et al., 1994). Briefly, under pentobarbital (40 mg/kg intraperitoneally) anaesthesia a Gregory-type gastric cannula was implanted in the forestomach. An indwelling catheter (PE-50) was inserted into the jugular vein. Both cannulas were tunnelled to the neck under the skin. Two other catheters (PE-50) were used to cannulate the bile-pancreatic duct at its entry into the duodenum, and to insert a cannula into the duodenum 2 cm distal to the pylorus. These catheters were exteriorised through the abdominal wall. After surgery, the animals were placed into Bollman-type cages. Bile-pancreatic and duodenal cannulas were connected between experiments in order to recirculate bile-pancreatic juice. Rats received lactated Ringer's solution at 0.8 ml/h intravenously and were fed intragastrically with a semi-elemental liquid diet (daily 2 ml/h for 10 h, 1 ml = 1 kcal, Pepti-2000 LF, EGIS-Nutricia, Budapest) through the gastric cannula. Experiments were carried out between the 3rd and 7th postoperative days, starting 8–10 h after completion of intragastric feeding. Bile-pancreatic juice was collected in 30-min periods. Pre-collected donor bile-pancreatic juice, pooled and diluted 1:1 with 0.15 M NaCl, was infused into the duodenum at 2.0 ml/h. During bile-pancreatic juice diversion, 0.15 M NaCl was infused into the duodenum at the same rate. Each experiment started with three 30-min basal periods, and basal secretion was regarded as the average of the second and third periods. When rats received different treatments in a given experiment, each rat received each treatment once on different days, and the order of the treatments was randomised within each experiment. Individual rats did not necessarily receive all treatments in a given set of experiments. The volume of bile-pancreatic juice was measured gravimetrically. Amylase activity was measured according to the method of Bernfeld (Bernfeld, 1955), using maltose as a standard. Amylase output was calculated and expressed as units per 30-min collection period (1 U = 1 μmol maltose/min).

In the first experiment, the effect of deramciclane and lorglumide on bile-pancreatic juice diversion-stimulated amylase secretion was investigated. Six different treatment groups were formed ($n = 7–10$): group 1, vehicle (1% methylcellulose in saline, 0.3 ml/rat i.v.); group 2, bile-pancreatic juice diversion from the duodenum; group 3, diversion + lorglumide (10 mg/kg); group 4, diversion + 3 mg/kg deramciclane; group 5, diversion + 10 mg/kg deramciclane; and group 6, diversion + 30 mg/kg deramciclane. Bile pancreatic juice diversion was continued during periods 4–7. Deramciclane and lorglumide were administered intravenously 5 min before the start of bile pancreatic juice diversion.

In another set of experiments the action of lorglumide and deramciclane was tested on CCK-8-induced pancreatic

amylase secretion ($n = 7–9$). A dose of CCK (200 pmol/kg h) that induces submaximal stimulation of pancreatic enzyme secretion (O'Rourke et al., 1989) was administered for 120 min after three basal periods. Vehicle, lorglumide (10 mg/kg) or deramciclane was given intravenously 5 min before CCK-8 (200 nmol/kg h i.v.). In this experiment only one dose of deramciclane (30 mg/kg) was tested. Otherwise, the experimental conditions were identical to those of the previous experiments.

2.2. Trophic studies with the pancreas

Six groups of intact rats ($n = 10–10$; initial body weight 150–170 g) were used. They were treated with the following compounds for five days: group 1 with saline, group 2 with camostate (200 mg/kg, once daily, intragastrically by gavage), group 3 with camostate and 3 mg/kg deramciclane, group 4 with camostate and 10 mg/kg deramciclane, group 5 with camostate and 30 mg/kg deramciclane, and group 6 with camostate + lorglumide (10 mg/kg). Deramciclane and lorglumide were administered intraperitoneally twice daily, 15 min before and 3 h after the proteinase inhibitor. Animals were killed after an overnight fast on the sixth day.

The pancreas was carefully trimmed free of fat, mesentery and lymph nodes, weighed, and homogenized in buffer (pH 8.0) of the following composition (mM): Tris–HCl 100, KCl 100, and $CaCl_2$ 20. Trypsin and amylase activities were then measured in the homogenate. Amylase activity was measured as described above (Bernfeld, 1955), while trypsin was estimated by a spectrophotometric method (Hummel, 1959) after activation of trypsinogen (Solomon et al., 1978). The tissue DNA content was determined by the method of Labarca and Paigen (Labarca and Paigen, 1980). Calf thymus DNA was used as a standard.

2.3. Gastric emptying

Under pentobarbital (40 mg/kg i.p.) anaesthesia a Gregory-type gastric cannula was implanted in the forestomach of rats (Varga et al., 1995; Varga and Scarpignato, 1996). An indwelling catheter (PE-50) was inserted into the jugular vein, and another cannula (PE-90) was inserted into the duodenum 2 cm distal to the pylorus. Both cannulas were tunnelled to the neck under the skin. Experiments were started after at least one week of recovery, during which time the animals became adapted to restraint for some hours twice a week in Bollman-type cages. The duodenal cannulas were filled with petroleum jelly between experiments.

Before experiments, the animals were fasted overnight and placed in Bollman-type cages. After the gastric cannula was opened, the stomach was rinsed with warmed saline and the experiments were started after at least 30 min. A 0.9 M NaCl solution containing phenol red (0.6 g/l) was used as noncaloric liquid test meal. 3 ml of pre-warmed (37°C) test meal were slowly (20 s) instilled into the stomach via a plastic catheter passed through a rubber plug fixed to the gastric cannula. Five minutes later the cannula was opened by removing the plug and the remaining gastric content was collected by gravity in graduated tubes. The stomach was then rinsed with 3 ml saline and the washing solution was added to the recovered gastric content. The phenol red concentration in the mixture was then measured spectrophotometrically at 560 nm by adding 0.1 N NaOH and the total amount of the marker recovered from the stomach was calculated. The data for emptying are expressed as percentages of liquid emptied over 5 min.

In the first set of experiments, the effect of intralipid, in concentrations capable of releasing endogenous CCK (Hölzer et al., 1994), on the gastric emptying of liquids was assessed. To this end, 0.9% saline or intralipid (5, 10 or 20%) was perfused intraduodenally at a rate of 0.1 ml/h ($n = 6–7$). The total volume of perfusate was 1.5 ml. Perfusion started 10 min before instillation of the noncaloric meal and continued throughout the measurement of gastric emptying.

In the second set of experiments, the action of vehicle (1% methylcellulose in saline), deramciclane and lorglumide on the 20% intralipid-induced delay in gastric emptying was studied. Vehicle (3 ml/rat), deramciclane (3, 10 and 30 mg/kg) or lorglumide (10 mg/kg) was given intragastrically 30 min before the meal ($n = 7–10$).

In the third set of experiments, the action of lorglumide was tested on the CCK-8-induced delay in gastric emptying ($n = 7–7$). Vehicle or lorglumide (10 mg/kg, i.g.) was given intragastrically 30 min before the meal. The CCK-8 (10 nmol/kg h) infusion was started 10 min before instillation of the noncaloric meal and continued throughout the measurement of gastric emptying. In this experiment, the animals did not have a duodenal cannula, and the stomach was rinsed with 5 ml saline (instead of 3 ml) during collection of the fluid remaining in the stomach. Otherwise, the experimental conditions were identical to those of the previous experiments.

2.4. Gallbladder emptying

Male NMRI mice (22–30 g; LabTech Kft, Charles River Europe) were fasted for 24 h before the experiments, but tap water was available ad libitum. The animals were randomly divided into 4–6 groups (8–12 animals/group) on each experimental day. They were treated with the test compound or the vehicle p.o. 45 min before either oral administration of 0.5 ml 30% freshly prepared egg yolk suspended in 0.4% methylcellulose solution or subcutaneous injection of CCK-8. Control animals were treated

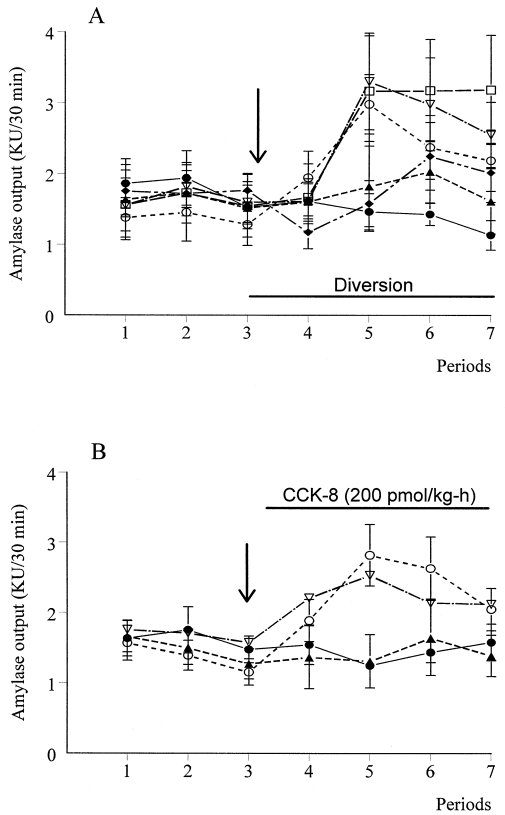

Fig. 1. Effect of deramciclane and lorglumide on bile-pancreatic juice diversion- and exogenous CCK-8-stimulated pancreatic amylase secretion in rats. (A) Bile-pancreatic juice diversion. The antagonists and saline were given after three basal collection periods as indicated by the arrow; Saline (●); VEH (○) = vehicle; 3 DER (▽), 10 DER (□) and 30 DER (◆) = deramciclane at 3, 10 and 30 mg/kg doses, respectively; 10 LOR (▲) = lorglumide at 10 mg/kg. Values are means ± S.E.M.; (n = 7–10). (B) CCK-8 stimulation. The antagonists and saline were given after three basal collection periods as indicated by the arrow; Saline (●); VEH (○) = vehicle; 30 DER (▽) = deramciclane at 30 mg/kg; 10 LOR (▲) = lorglumide at 10 mg/kg. Values are means ± S.E.M. (n = 7–9).

with vehicle instead of egg yolk or CCK-8. Mice were killed by cervical dislocation 15 min later and the gallbladder was quickly removed and weighed.

Three separate experiments were performed. In the first experiment, the dose–response effect of deramciclane (0.3–30 mg/kg) on egg yolk-stimulated gallbladder emptying was tested. In the second one, the inhibitory action of lorglumide (1–10 mg/kg) was investigated under similar conditions. Finally, in the third experiment, the effects of deramciclane (30 mg/kg) and lorglumide (10 mg/kg) were studied on gallbladder emptying stimulated by 1 μg/kg CCK-8.

2.5. Evaluation of data

All data are presented as means ± S.E.M. In secretory experiments cumulative amylase output over basal secretion was calculated during the first 90 min after treatment for each rat. Basal secretion was regarded as the average of the second and third collection periods. One-way analysis of variance (ANOVA) followed by Student–Newman–Keuls multiple comparisons test were used to evaluate statistical significance. This analysis was performed by using the InStat program (GraphPad Software, San Diego, CA) running on an IBM PC.

2.6. Drugs

CCK-8 (Research Plus, Bayonne, NJ) was dissolved in saline containing 0.2% bovine serum albumin (Sigma St. Louis, MO). Intralipid (20%, Kabivitrum) was diluted in physiological saline. Camostate (compound marked FOY-305, Ono Pharmaceuticals, Osaka, Japan) and lorglumide (RBI) were dissolved in saline. Deramciclane (EGIS Pharmaceuticals) was suspended daily in a solution containing 0.4–1% methylcellulose (Methocell F4M, Dow Chemical, USA). Fresh solutions of each compound were prepared each experimental day.

3. Results

3.1. Pancreatic enzyme secretion in rats

Diversion of bile-pancreatic juice from the duodenum resulted in a significant increase in pancreatic amylase

Table 1
Effect of deramciclane and lorglumide on bile-pancreatic juice diversion-stimulated pancreatic amylase secretion in rats

	Mean ± S.E.M.	Difference vs. saline	Difference vs. diversion alone
Saline	−0.7 ± 0.659	–	–
Diversion	3.188 ± 0.815	$P < 0.05$	–
Diversion + 10 mg/kg LOR	0.579 ± 0.929	NS	$P < 0.05$
Diversion + 3 mg/kg DER	2.763 ± 0.827	$P < 0.05$	NS
Diversion + 10 mg/kg DER	3.092 ± 0.913	$P < 0.05$	NS
Diversion + 30 mg/kg DER	−0.233 ± 0.913	NS	$P < 0.05$

Diversion = diversion of bile-pancreatic juice from the duodenum; LOR = lorglumide, DER = deramciclane; NS = not significant.
Data represent cumulative amylase output (minus basal) for collection periods 4 through 6.
Basal secretion was determined as the average of the 2nd and 3rd periods (depicted in Fig. 1A).

Table 2
Effect of deramciclane and lorglumide on CCK-stimulated pancreatic amylase secretion in rats

	Mean ± S.E.M.	Difference vs. saline	Difference vs. CCK-8 alone
Saline	−0.813 ± 0.295	–	–
CCK-8	3.516 ± 0.753	$P < 0.01$	–
CCK-8 + 10 mg/kg LOR	0.154 ± 1.021	NS	$P < 0.01$
CCK-8 + 10 mg/kg DER	1.947 ± 0.809	$P < 0.05$	NS

LOR = lorglumide, DER = deramciclane; CCK-8: 200 pmol/kg h i.v. infusion; NS = not significant.
Data represent cumulative amylase output (minus basal) for collection periods 4 through 6.
Basal secretion was determined as the average of the 2nd and 3rd periods (depicted in Fig. 1B).

secretion (cumulative secretory response: 3.19 ± 0.81 kU/90 min) in rats (Fig. 1A and Table 1). The two lower doses of deramciclane (3 and 10 mg/kg) did not modify this effect of diversion. On the contrary, 30 mg/kg deramciclane and 10 mg/kg lorglumide, given intravenously, almost completely counterbalanced the secretory response to diversion (cumulative secretory responses: −0.23 ± 0.59 KU/90 min, and 0.68 ± 0.92 kU/90 min, respectively; Fig. 1A and Table 1).

As expected, exogenous CCK (200 pmol/kg h) also stimulated pancreatic enzyme secretion. In this case, administration of 10 mg/kg lorglumide completely abolished the stimulatory action of the peptide, while 30 mg/kg deramciclane did not significantly modify the effect of CCK (Fig. 1B and Table 2).

3.2. Pancreatic growth in rats

All tissue parameters given are normalised to unit body weight. As previously described, chronic administration of camostate increased the weight of the pancreas, as well as the pancreatic DNA content (Fig. 2). Lower doses of deramciclane (3 and 10 mg/kg) did not modify these effects of camostate. At a dose of 30 mg/kg, deramciclane and lorglumide inhibited the camostate-induced increase in pancreatic weight and DNA content (Fig. 2). Camostate treatment also stimulated the pancreatic trypsin content

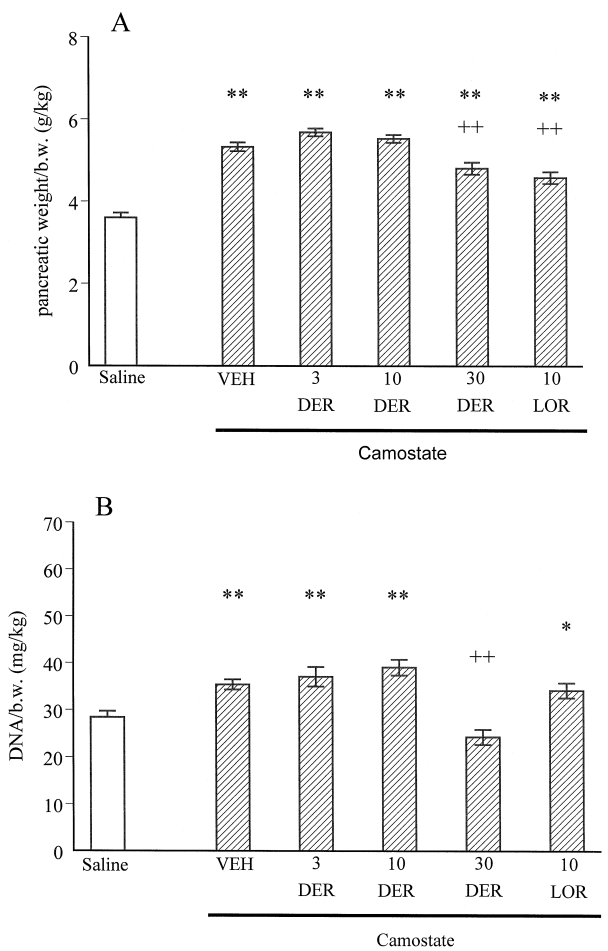

Fig. 2. Effect of deramciclane and lorglumide on 5-day camostate treatment-evoked changes in pancreatic weight (A) and tissue DNA content (B) in rats. Black columns: camostate treatment; VEH = vehicle; 3 DER, 10 DER and 30 DER = deramciclane at 3, 10 and 30 mg/kg doses, respectively; 10 LOR = lorglumide at 10 mg/kg. Values are means ± S.E.M. ($n = 10–10$). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. Saline; $^{++}P < 0.01$ vs. camostate alone (VEH).

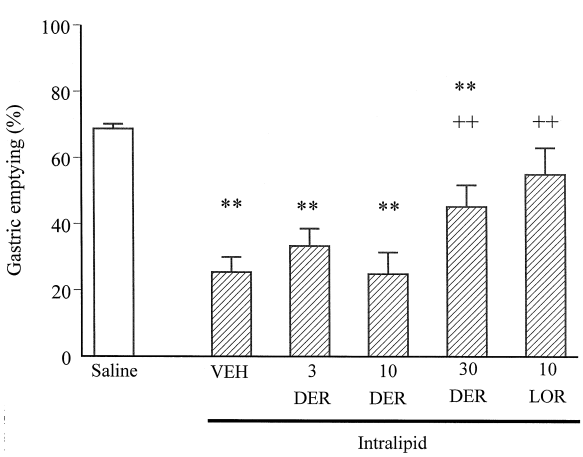

Fig. 3. Effect of deramciclane and lorglumide on intralipid-evoked acceleration of gastric emptying in rats. Black columns: 20% Intralipid infusion; VEH = vehicle; 3 DER, 10 DER and 30 DER = deramciclane at 3, 10 and 30 mg/kg doses, respectively; 10 LOR = lorglumide at 10 mg/kg. Values are means ± S.E.M. ($n = 7–10$). $^{**}P < 0.01$ vs. Saline; $^{++}P < 0.01$ vs. intralipid alone (VEH).

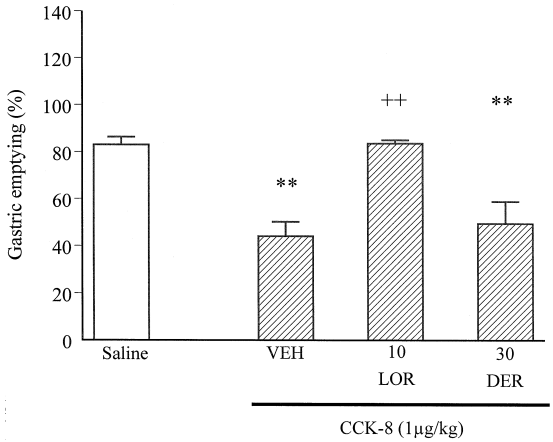

Fig. 4. Effect of deramciclane and lorglumide on CCK-evoked gastric emptying. Black columns = CCK-8 in 1 μg/kg dose; VEH = vehicle; 30 DER = deramciclane 30 mg/kg; 10 LOR = lorglumide 10 mg/kg. Values are means ± S.E.M. ($n = 7–7$). $^{**}P < 0.01$ vs. Saline; $^{++}P < 0.01$ vs. CCK-8 alone (VEH).

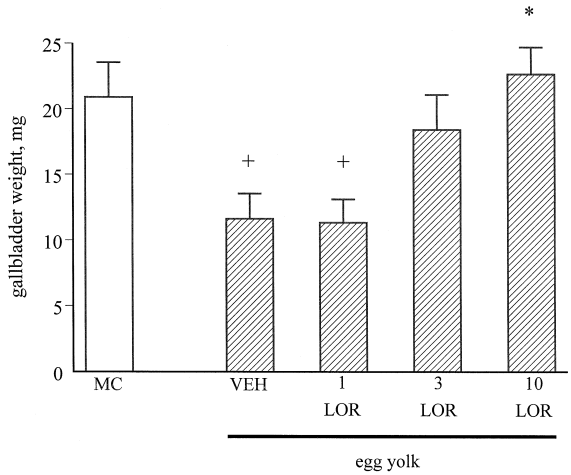

Fig. 6. Effect of lorglumide on egg yolk-evoked acceleration of gallbladder emptying in mice. Black columns: egg yolk i.g.; VEH = vehicle; 1–10 LOR = lorglumide at 1–10 mg/kg doses, respectively. Values are means ± S.E.M. ($n = 8–12$). $^{*}P < 0.05$ vs. Saline; $^{+}P < 0.05$ vs. egg yolk alone (VEH).

($P < 0.01$), an effect that was abolished by both 30 mg/kg deramciclane and 10 mg/kg lorglumide. No significant changes were observed, however, in pancreatic amylase content among the different experimental groups (data not shown).

3.3. Gastric emptying in rats

Five min after instillation of physiological saline into the rat stomach, 32 ± 4% of the fluid was recovered. When intralipid (5, 10 and 20%) was given intraduodenally together with the test meal, it delayed gastric emptying in a concentration-dependent manner (data not shown). For further studies, we used a 20% intralipid infusion because this dose induced the most pronounced effect.

In the next set of experiments, 20% Intralipid infusion into the duodenum resulted in a delay in gastric emptying (Fig. 3). The two lower doses of deramciclane (3 and 10 mg/kg) did not affect this action of intralipid. On the contrary, 30 mg/kg deramciclane and lorglumide, given intragastrically, significantly inhibited the delay induced by the lipid. It is worth mentioning, however, that none of these compounds was able to completely counterbalance the intralipid-induced delay in gastric emptying (Fig. 3).

Exogenous CCK (10 nmol/kg h) also induced a delay in the emptying of the noncaloric test meal. In this case, administration of 10 mg/kg lorglumide completely abolished the action of the peptide while 30 mg/kg deramciclane did not modify it (Fig. 4).

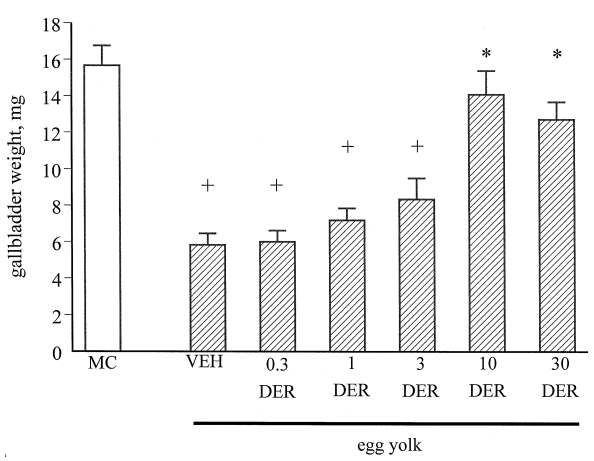

Fig. 5. Effect of deramciclane on egg yolk-evoked acceleration of gallbladder emptying in mice. Black columns: egg yolk i.g.; VEH = vehicle; 0.3–30 DER = deramciclane at 0.3–30 mg/kg doses, respectively. Values are means ± S.E.M. ($n = 8–12$). $^{*}P < 0.05$ vs. Saline; $^{+}P < 0.05$ vs. egg yolk alone (VEH).

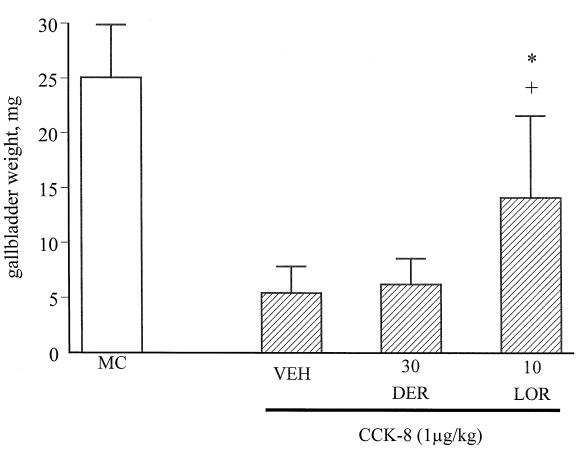

Fig. 7. Effect of deramciclane and lorglumide on CCK-evoked acceleration of gallbladder emptying in mice. Black columns = CCK-8 at 1 μg/kg dose; VEH = vehicle; 30 DER = deramciclane at 30 mg/kg; 10 LOR = lorglumide at 10 mg/kg. Values are means ± S.E.M.; ($n = 9–12$). $^{*}P < 0.05$ vs. saline; $^{+}P < 0.05$ vs. CCK-8 alone (VEH).

3.4. Gallbladder emptying in mice

Intragastric egg yolk administration decreased gallbladder weight in fasted mice from 15.7 ± 1.5 to 5.9 ± 0.9 mg. Deramciclane dose dependently antagonised the effect of egg yolk and the gallbladder weight was restored to the level of the vehicle-treated group after doses of 10 and 30 mg/kg (Fig. 5). Lorglumide had similar effects at somewhat lower doses (Fig. 6). Exogenous CCK-8 (1 μg/kg) accelerated the emptying of gallbladders in fasted mice also. The effect of CCK-8 was partially antagonised by lorglumide at 10 mg/kg, but deramciclane at 30 mg/kg was without an effect (Fig. 7).

4. Discussion

In the present investigation, we compared the effect of two bioactive substances, the serotonin receptor antagonist deramciclane and the CCK_A receptor antagonist lorglumide, on exogenous and endogenous CCK-stimulated pancreatic amylase secretion and pancreatic growth, as well as on modulation of the emptying of the stomach and the gallbladder.

Pancreatic function in rats is known to be regulated by a negative feed-back loop that is essentially controlled by inhibition of CCK release by intraduodenal protease activity (Lu et al., 1989; Miyasaka et al., 1989; Li et al., 1995). Indeed, we found that the diversion of bile-pancreatic juice from the duodenum induced a marked pancreatic secretory response in rats. Lorglumide, at a dose (10 mg/kg) which blocked CCK-stimulated enzyme secretion, abolished the secretory response to diversion of bile-pancreatic juice, indicating that CCK-like peptides mediate this process in the pancreas. Our results are in agreement with previous studies which suggest that the pancreatic secretory response to diversion is mediated mainly by CCK (Louie et al., 1986). The two lower doses of deramciclane did not affect significantly the diversion-induced release of amylase. The highest dose of the compound, however, significantly inhibited the effect of diversion, and the magnitude of inhibition was similar to that evoked by CCK receptor blockade with lorglumide. Under our experimental conditions, CCK-8 infusion significantly increased pancreatic amylase secretion. This action was completely blocked by the CCK_A receptor antagonist. Deramciclane, applied at the same dose that inhibited the actions of endogenous CCK, did not significantly affect the amylase response to exogenous CCK. Our findings suggest that the site of action of deramciclane is not at the level of pancreatic CCK_A receptors. This is in good agreement with recent reports that the 5-HT_2 receptor antagonist ketanserin is able to inhibit the pancreatic secretory response to endogenous CCK stimulation in rats (Li and Owyang, 1996a; Li et al., 1996b).

Göke et al. (1986) first showed that chronic stimulation of endogenous CCK release by camostate, a proteinase inhibitor, stimulated pancreatic enzyme secretion and induced pancreatic hypertrophy and hyperplasia. Subsequent studies have confirmed the initial observations. In our study, the ability of lorglumide to reduce the trophic effect of the proteinase inhibitor is in accordance with data from previous investigations showing that CCK_A receptor antagonists (namely devazepide, lorglumide and dexloxiglumide) are able to counterbalance the pancreatic secretory and growth-promoting effects of exogenous or endogenous CCK in rodents (Wisner et al., 1988; Schmidt et al., 1989; Scarpignato et al., 1989; Douglas et al., 1990; Varga et al., 1998). The two lower doses of deramciclane did not affect the pancreatic growth induced by camostate. At the same time, the highest dose of the compound significantly inhibited the trophic action of the proteinase inhibitor, and the magnitude of inhibition was similar to that evoked by CCK receptor blockade.

In both animals and humans, inhibition of gastric emptying by CCK and related peptides involves a drop in intragastric pressure, due to the relaxation of the proximal stomach, and contraction of the antropyloric region, where the peptide decreases the motility index and the basal electric rhythm (Bertaccini, 1982). Intestinal lipid has been shown to inhibit gastric emptying, and this process is mediated in part by CCK (Mayer, 1994; Hölzer et al., 1994). We also found that intraduodenal lipid infusion delayed gastric emptying. This action of lipid was inhibited, but not completely blocked, by the CCK_A receptor antagonist lorglumide. Lorglumide, applied at the same dose, completely inhibited the actions of a high dose of exogenous CCK-8 (inducing a similar delay in emptying as lipid infusion). Therefore, our data are in line with the previous observation that the effect of intralipid on gastric motility is mediated by other mediators as well (Hölzer et al., 1994). The two lower doses of deramciclane did not affect significantly the CCK-induced delay. The highest dose of the compound significantly inhibited the action of intralipid, and the degree of inhibition was similar to that evoked by CCK receptor blockade. Whether this action of deramciclane on gastric emptying is due to its inhibitory effect of CCK release, or to other unrelated mechanisms, needs further investigation.

The stimulatory effect of egg yolk on gallbladder emptying has been demonstrated in several studies (Makovec et al., 1987a,b; Gully et al., 1993), and it has been shown that this stimulation is mediated by activation of CCK_A receptors (Makovec et al., 1987a,b; Gully et al., 1993). In the present work, we confirmed that both exogenous CCK and endogenous CCK stimulate gallbladder motility. We also found that endogenous CCK-mediated actions were inhibited by both deramciclane and lorglumide in a dose-dependent manner. In contrast, exogenous CCK-mediated actions were inhibited only by lorglumide but not by the serotonin receptor antagonist. Therefore, the effect of the

serotonin receptor antagonist in mice shows characteristics similar to those seen in rats on pancreatic function and gastric emptying. In addition, our data suggest that mice are more sensitive to deramciclane administration than rats, because the compound was active at 10 mg/kg in mice and only at 30 mg/kg in rats.

In conclusion, our results suggest that both the 5-$HT_{2A/2C}$ receptor antagonist deramciclane and the CCK_A receptor antagonist lorglumide are able to modulate four physiological functions that involve the release of endogenous CCK in rodents: pancreatic enzyme secretion, pancreatic growth, gastric emptying and gallbladder motility. The stimulation of pancreatic amylase secretion by exogenous CCK administration was inhibited by lorglumide, but not by deramciclane. Therefore, the mechanism of inhibitory action of deramciclane probably involves the inhibition of endogenous CCK release. Furthermore, this effect is mediated via 5-HT_{2A} receptors, as was also suggested by Li and Owyang (1996a) and Li et al. (1996b). In addition, the compound may also inhibit cholinergic-peptidergic transmission, which is also important for maintaining basal pancreatic secretion and mediating pancreatic secretory responses to different stimuli (Louie et al., 1986; Lu et al., 1989; Miyasaka et al., 1989; Li et al., 1995). Although the exact mechanism of action of deramciclane is not known, our observations provide evidence that serotoninergic pathways are involved in the regulatory actions of CCK in the gastrointestinal tract.

Acknowledgements

This study was supported in part by grants from the Hungarian National Research Fund (T-022401) and from the Health Research Council Fund (ETT-193/1996 02).

References

Berger, Z., Fehér, E., 1997. Degeneration of intrapancreatic nerve fibers after chronic alcohol administration in mice. Int. J. Pancreatol. 21 (2), 165–171.

Bernfeld, P., 1955. Amylases, alpha end beta. In: Colowick, S.P., Kaplan, N.O. (Eds.), Methods in Enzymology, Vol. I. Academic Press, New York, 149–158.

Bertaccini, G., 1982. Gastrointestinal hormones. In: Bertaccini, G. (Ed.), Handbook of Experimental Pharmacology, Vol. 59. Springer, Berlin, 11–83.

Bonhaus, D.W., Bach, C., DeSouza, A., Salazar, F.H., Matsuoka, B.D., Zuppan, P., Chan, H.W., Eglen, R.M., 1995. The pharmacology and distribution of human 5-hydroxytryptamine2B(5-HT2B) receptor gene products: comparison with 5-HT2A and 5-HT2C receptors. Br. J. Pharmacol. 115 (4), 622–628.

Chey, W.Y., 1993. Hormonal control of pancreatic exocrine secretion. In: Go, V.L.W. et al. (Eds.), The Pancreas: Biology, Pathobiology and Disease. Raven Press, New York, 117–141.

Dockray, G.J., 1989. Comparative neuroendocrinology of gut peptides. In: Makhlouf, G.M. (Ed.), The Gastrointestinal System, Vol. 2. The American Physiological Society, New York, 133–170.

Douglas, B.R., Woutersen, R.A., Jansen, J.B., Rovati, L.C., Lamers, C.B., 1990. Comparison of the effect of lorglumide on pancreatic growth stimulated by camostate in rat and hamster. Life Sci. 46, 281–286.

Fölsch, U.R., 1984. Regulation of pancreatic growth. Clin. Gastroenterol. 13, 679–699.

Gacsályi, I., Gyertyán, I., Petocz, L., Budai, Z., 1988. Psychopharmacology of a new anxiolytic agent EGYT-3886. Pharmacol. Res. Commun. 20 (1), 115–116.

Gacsályi, I., Gigler, G., Szabados, T., Kovács, A., Vasar, E., Lang, A., Mannistö, P.T., 1996. Different antagonistic activity of deramciclane (EGIS-3886) on peripherial and central 5-HT2 receptors. Pharm. Pharmacol. Lett. 6 (2), 82–85.

Gacsályi, I., Schmidt, E., Gertyán, I., Vasar, E., Lang, A., Haapalinna, A., Fekete, M., Hietala, J., Syvalahati, E., Mannistö, R.T., 1997. Receptor binding profile and anxiolytic activity of deramciclane (EGIS-3886) in animal models. Drug Dev. Res. 40 (4), 333–348.

Göke, B., Printz, H., Koop, I., Rausch, U., Richter, G., Arnold, R., Adler, G., 1986. Endogenous CCK release and pancreatic growth in rats after feeding a proteinase inhibitor (camostate). Pancreas 1, 509–515.

Green, T., Dimaline, R., Peikin, S., Dockray, G.J., 1988. Action of the cholecystokinin antagonist L-364,718 on gastric emptying in the rat. Am. J. Physiol. 255, G685–G689.

Gully, D., Frehel, D., Marcy, C., Spinazze, A., Lespy, L., Neliat, G., Maffrand, J.P., Le Fur, G., 1993. Peripheral biological activity of SR 27897: a new potent non-peptide antagonist of CCK(A) receptors. Eur. J. Pharmacol. 232, 13–19.

Hölzer, H.H., Turkelson, C.M., Solomon, T.E., Raybould, H.E., 1994. Intestinal lipid inhibits gastric emptying via CCK and a vagal capsaicin-sensitive afferent pathway in rats. Am. J. Physiol. 267, G625–G629.

Hummel, B.C., 1959. A modified spectrophotometric method for determination of chymotrypsin trypsin and thrombin. Can. J. Biochem. Physiol. 37, 1393–1399.

Kirchgessner, A.L., Liu, M.T., Raymond, J.R., Gershon, M.D., 1996. Identification of cells that express 5-hydroxytryptamine1A receptors in the nervous systems of the bowel and pancreas. J. Comp. Neurol. 364 (3), 439–455.

Kuemmerle, J.F., Murthy, K.S., Grider, J.R., Martin, D.C., Makhlouf, G.M., 1995. Coexpression of 5-HT4 receptors coupled to distinct signaling pathways in human intestinal muscle cells. Gastroenterology 109 (6), 1791–1800.

Labarca, C., Paigen, K., 1980. A simple, rapid and sensitive DNA assay procedure. Anal. Biochem. 102, 344–352.

Li, Y., Hao, Y., Owyang, C., 1995. Evidence for autoregulation of cholecystokinin secretion during diversion of bile pancreatic juice in rats. Gastroenterology 109, 231–238.

Li, Y., Owyang, C., 1996a. Peptone stimulates CCK-releasing peptide secretion by activating intestinal submucosal cholinergic neurons. J. Clin. Invest. 97 (6), 1463–1470.

Li, Y., Hoa, Y., Owyang, C., 1996b. CCK-releasing peptide mediates oleic acid stimulated secretion of CCK: involvement of intestinal submucosal cholinergic neurons. Regul. Peptides 64, 110.

Liddle, R.A., 1994. Cholecystokinin. In: Walsh, J.H., Dockray, G.J. (Eds.), Gut Peptides: Biochemistry and Physiology. Raven Press, New York, 175–216.

Louie, D.S., May, D., Miller, P., Owyang, C., 1986. Cholecystokinin mediates feedback regulation of pancreatic enzyme secretion in rats. Am. J. Physiol. 250, G252–G259, Gastrointest. Liver Physiol. 13.

Lu, L., Louie, D., Owyang, C., 1989. A cholecystokinin releasing peptide mediates feedback regulation of pancreatic secretion. Am. J. Physiol. 256, G430–G435.

Makovec, F., Bani, M., Cereda, R., Chiste, R., Pacini, M.A., Revel, L., Rovati, L.A., Rovati, L.C., Setnikar, I., 1987a. Pharmacological properties of lorglumide as a member of a new class of cholecystokinin antagonists. Arzneimittelforschung 37 (II), 1265–1268.

Makovec, F., Bani, M., Cereda, R., Chiste, R., Pacini, M.A., Revel, L.,

Rovati, L.C., 1987b. Antispasmodic activity on the gallbladder of the mouse of CR 1409 (lorglumide) a potent antagonist of peripheral CCK. Pharmacol. Res. Com. 19, 41–51.

Mayer, E.A., 1994. The physiology of gastric storage and emptying. In: Johnson, L.R. et al. (Eds.), Physiology of the Gastrointestinal Tract, 3rd edn. Raven Press, New York, 929–976.

Miller, L.J., 1984. Characterization of cholecystokinin receptors on human gastric smooth muscle tumors. Am. J. Physiol. 247, G402–G410.

Miyasaka, K., Guan, D., Liddle, R.A., Green, G.M., 1989. Feedback regulation by trypsin: evidence for intraluminal CCK releasing peptide. Am. J. Physiol. 256, G175–G181.

O'Rourke, M.F., Reidelberger, R.D., Solomon, T.E., 1989. Effect of CCK antagonist L 364718 on meal-induced pancreatic secretion in rats. Am. J. Physiol. 258, G179–G184, Gastrointest. Liver Physiol. 21.

Reidelberger, R.D., Varga, G., Liehr, R.M., Castellanos, D.A., Rosenquist, G.L., Wong, H.C., Walsh, J.H., 1994. Cholecystokinin suppresses food intake by a non-endocrine mechanism in rats. Am. J. Physiol. 267, R901–R908.

Scarpignato, C., Varga, G., Dobronyi, I., Papp, M., 1989. Effect of a new potent CCK antagonist, lorglumide, on caerulein- and bombesin-induced pancreatic secretion and growth in the rat. Br. J. Pharmacol. 96, 661–669.

Scarpignato, C., Varga, G., Corradi, C., 1993. Effect of CCK and its agonists on gastric emptying. J. Physiol. 87, 291–300.

Schmidt, W.E., Stockmann, F., Choudhury, A.R., Wilms, H.M., Siegel, E.G., Nustede, R., Folsch, U.R., Creutzfeldt, W., 1989. Influence of CCK antagonist L-364,718, pancreastatin (33-49) and a somatostatin analogue on camostate-induced rat pancreatic hypertrophy. Digestion 44, 105–116.

Sha, L., Ou, L.L., Miller, S.M., Ma, R., Szurszewski, J.H., 1996. Cat pancreatic neurons: morphology, electrophysiological properties, and responses to 5-HT. Pancreas 13 (2), 111–124.

Solomon, T.E., 1981. Regulation of exocrine pancreatic cell proliferation and enzyme synthesis. In: Johnson, L.R. et al. (Eds.), Physiology of the Gastrointestinal Tract. Raven Press, New York, 873–892.

Solomon, T.E., Petersen, H., Elashoff, J., Grossman, T.E., 1978. Interaction of caerulein and secretin on pancreatic size and composition in rat. Am. J. Physiol. 235, E714–E719.

Varga, G., Reidelberger, R.D., Lieh, R.-M., Bussjaeger, L.J., Coy, D.H., Solomon, T.E., 1991. Effects of potent bombesin antagonist on exocrine pancreatic secretion in rats. Peptides 12, 493–497.

Varga, G., Liehr, R.-M., Scarpignato, C., Coy, D.H., 1995. Distinct receptors mediate gastrin-releasing peptide and neuromedin B induced delay of gastric emptying of liquids in rats. Eur. J. Pharmacol. 286, 109–112.

Varga, G., Scarpignato, C., 1996. Camostate and caerulein-induced delay of gastric emptying in the rat: effect of CCK-receptor antagonists. Eur. J. Pharmacol. 306, 153–159.

Varga, G., Kisfalvi, K., D'Amato, M., Scarpignato, C., 1998. Different actions of CCK on pancreatic and gastric growth in the rat: effect of CCK-A receptor blockade. Br. J. Pharmacol. 124, 435–440.

Wisner, J.R., McLaughlin, R.E., Rich, K.A., Ozawa, S., Renner, I.G., 1988. Effects of L-364,718, a new cholecystokinin receptor antagonist, on camostate-induced growth of the rat pancreas. Gastroenterology 94, 109–113.

Woodruff, G.N., Hughes, J., 1991. Cholecystokinin antagonists. Annu. Rev. Pharmacol. Toxicol. 31, 469–501.