Belg. J. Zool. — Volume 125 (1995) — issue 1 — pages 251-259 — Brussels 1995

GALLBLADDER CONTRACTIONS IN CHICKENS, GUINEA PIGS AND MICE FOLLOWING TREATMENT WITH SIMMONDSIN OR CHOLECYSTOKININ

by

SABIEN VERMAUT (1), PAUL BUSSELEN (2), STUART SPENCER (3), MARNIX COKELAERE (2), GERDA FLO (2) and EDDY DECUYPERE (1)
(1) Laboratory for Physiology and Immunology of Domestic Animals, Kardinaal Mercierlaan 92, B-3001 Heverlee (Belgium)
(2) Disciplinary Research Center, E. Sabbelaan 53, B-8500 Kortrijk (Belgium)
(3) AgResearch, Ruakura Agricultural Centre, East Street, Private Bag 3123, Hamilton, New Zealand

SUMMARY

The jojoba plant (*Simmondsia chinensis*) is a native oilseed shrub of the Sonoran desert. Feeding jojoba meal — a byproduct of the oil extraction — results in reduced food intake in rats, chickens, ewes and rodents. This inhibitory effect is due to the presence of a glycoside, simmondsin. Its satiating effect is either by a stimulation of the secretion of cholecystokinin (CCK) as a satiating factor or by acting as a CCK-agonist itself. In this study, the effect of CCK and simmondsin on gallbladder contraction *in vivo* and *in vitro* was tested using mice, guinea pigs and chickens as experimental models.

In vivo, total gallbladder contraction was observed after i.p. injection of a high dose of (mammalian) CCK-8 in mice, while no contraction at all was observed in chickens. *In vitro* however, the isolated gallbladders from both mice and chickens contracted, but the treshold for gallbladder contraction induced by CCK-8 was higher in chickens than in mice. Moreover, the contraction pattern was continuously pulsatile and oscillating in chickens while a single, large contraction was observed in mice. Simmondsin had no direct contractile effect on gallbladder strips in mouse, chicken or guinea pig. This suggests that it probably has its satiating effect by stimulating release of endogenous CCK. It was further demonstrated that simmondsin stimulated duodenal CCK secretion in guinea pig since gallbladder contractions *in vitro* were intensified, when the latter was flooded with duodenal secretions collected after *in vitro* treatment with simmondsin. Moreover, in this *in vitro* system a CCK-A antagonist (devapezide) neutralised the effect of duodenal CCK, obtained after simmondsin stimulation.

Keywords : gallbladder, CCK, simmondsin, chicken, mice, guinea pig.

INTRODUCTION

The jojoba plant (*Simmondsia chinensis*) is a native oilseed shrub of the Sonoran desert (BOOTH *et al.*, 1974). Feeding jojoba meal — a byproduct of the oil extraction — results in reduced food intake in rats (BOOTH *et al.*, 1974 ; COKELAERE *et al.*, 1992), chickens (NGOU NGOUPAYOU *et al.*, 1982 ; ARNOUTS *et al.*, 1993), ewes (MANOS *et al.*, 1986) and rodents (SHERBROOKE *et al.*, 1976). This inhibitory effect is due to the presence of a 2-cyanomethylene-3-hydroxy-4,5-dimethoxycyclohexyl - B-D-glycoside, called simmondsin (ELLIGER *et al.*, 1973, 1974a, 1974b ; BOOTH *et al.*, 1974). It appears that simmondsin exerts its satiating effect by a stimulation of the cholecystokinin (CCK) system, since its effect is inhibited by the simultaneous administration of devapezide, a peripheral CCK-receptor antagonist (COKELAERE *et al.*, in press). It is, however, not clear if simmondsin induces satiation by stimulating release of CCK, or by acting directly as a CCK-agonist. In order to discriminate between these two possibilities, the effect of simmondsin has been compared with the effects of CCK on gallbladder contractions *in vivo* and *in vitro*.

MATERIAL AND METHOD

Drugs used

Hexane-extracted jojoba meal was re-extracted with acetone in a Soxhlet extractor for 8 h. After crystallization, the mixture of simmondsin and its analogues, diluted in water, was put on a Sephadex column for preparative chromatography. The simmondsin fraction was purified by TLC (silica gel TLC plates Merck no. 5553 ; solvent, ethylacetate/ethanol 70 :30 with 10 % H_2SO_4) monitored by IR spectrophotometry (VAN BOVEN *et al.*, 1993).

Sulphated CCK octapeptide (CCK-8) was purchased from Sigma (St. Louis, USA) and devapezide (MK-329), a specific CCK-A-receptor antagonist, was donated by Merck Sharp and Dohme Research Laboratories. All solutions were prepared daily prior to injection. Before each injection they were diluted with physiological saline (0.9 % NaCl) to the required concentration. For the *in vitro* work, solutions were added to Krebs phosphosaline buffer (KPS ; pH 7.4, 37° C).

Experiments

In vitro

Strips of gallbladder of mice (BALB\C), guinea pig or chicken (Hisex) were mounted in a superfusion bath and connected to an isometric force transducer. After ca. 60 min of perfusion with KPS, the preparations became spontaneously active and sensitive to low doses of CCK-8. The effects of increasing concentrations of CCK-8 (1 nM CCK-8 up to 100 nM CCK-8) and 10 mM simmondsin were therefore tested after ca. 80 min of superfusion.

To investigate the possible secretion of endogenous CCK, chicken duodenum was isolated, turned inside out and perfused with KPS (serosal solution) in a re-circulatory fashion. This re-iterative way was chosen to concentrate any product released by the gut. The outside (mucosa) was also continuously bathed in KPS, but separated from the inside circulation. After 30 minutes of perfusion the serosal perfusate was collected (solution A) and replaced with a new KPS, to which 10 mM simmondsin was added. Following a further 30 minutes perfusion the perfusate was collected (solution B).

The serosal solutions were then tested for their contracting activity on chicken gallbladder. Similar perfusates were collected after incubation with guinea pig duodenum and were tested on guinea pig gallbladder.

After 30 minutes the serosal solution B of the guinea pig was tested again in the presence of the specific CCK receptor antagonist devazepide. Devapezide was dissolved in DMSO (10 mg/ml) and then diluted in KPS in a concentration of 1 µg/ml. For control the same amount of DMSO without devazepide was tested.

In vivo

Standard gallbladder contraction assays were performed following the method described by MAKOVEC *et al.* (1987). Experiments were performed on 30 mice (CD1, 25 weeks, both sexes), weighing approximately 30 g. They received a standard diet and were fasted overnight (17 hours) before the experiments started. Water was freely available throughout. Five mice were injected intraperitoneally (i.p.) with 1.25 µg CCK-8/kg body weight (BW) in 80 µl saline. Five were injected i.p. with 1 g simmondsin/kg BW in 80 µl saline. Five were injected i.p. with 800 µg/kg BW MK-329, soluted in glycerol and PEG 400, diluted in 100 µl saline, followed 15 minutes later by an i.p. injection of 1.25 µg CCK-8/kg BW in 80 µl saline. For control i.p. injections, the same concentration of glycerol and PEG 400 diluted in 100 µl saline, followed by an injection of 80 µl saline, was tested in 5 mice. Fifteen minutes after injection, the mice were killed and their gallbladder was removed and weighed.

A similar procedure was repeated with 64 male chickens (Hisex, 3 weeks old), weighing approximately 160 g. Sixteen animals were injected i.p. with 6.25 µg CCK-8/kg BW in 500 µl saline, whereas 16 birds were injected (i.p.) with 1 g simmondsin/kg BW in 500 µl saline. Sixteen control animals received 500 µl saline only. The last 16 chickens received non treatment but were fed after the same starvation period. In each treatment gallbladders were removed at 15 or 30 minutes after the injection ($n = 8$ for each group).

The data were analyzed using analysis of variance followed by Duncan's multiple range test using the SAS program (SAS, 1985).

RESULTS AND DISCUSSION

Below 100 nM, CCK had no effect in eliciting *in vitro* gallbladder contraction in chicken (data not shown), whereas in mice (Fig. 1) and guinea pig (data not shown), doses as low as 1 nM were effective. DIMALINE *et al.* (1990) reported,

however, that the treshold dose of CCK for *in vitro* chicken or guinea pig gallbladder contraction was 0.1 nM. Moreover, in our study, the contraction pattern in chicken gallbladder was continuously pulsatile and oscillating, while a single, large contraction was observed in mice (Figs 1-2-3). This oscillating contraction pattern in chicken gallbladder contraction wasn't observed by DIMALINE *et al.* (1990). The sensitivity of the technique for measuring the contractions can be different, because the tissue bath techniques used by these authors were not reported.

In our *in vitro* studies there were initially no contractions in the gallbladder of mice, guinea pig and chicken before 60 minutes, after which spontaneous contractions started to appear. This might suggest that there is a basal CCK secretion by the gallbladder itself. It would appear that in the fresh tissue preparation *in vitro*, there is insufficient CCK to produce an effect, but after one hour of incubation enough endogenous CCK may have been produced to start contractions. Such a possibility is further supported by the finding that the CCK antagonist, devapezide, eliminates the spontaneous contractions. We know of no previous suggestions that the gallbladder itself may produce CCK.

Simmondsin, at 10 mM *in vitro*, had no direct contractile effect on gallbladder strips in mouse, chicken or guinea pig (Figs 1-2). This observation indicates that simmondsin does not act as a CCK agonist on mammalian or chicken gallbladder receptors. In guinea pig, however, a substance found in duodenal perfusates after stimulation with simmondsin (solution B), did stimulate guinea pig gallbladder contraction. These data suggest that simmondsin induces CCK release from guinea pig duodenum.

Gallbladder contractions could be seen prior to any application of simmondsin to the gut (Fig. 4). Simmondsin treatment however (Solution B), intensified these contractions, an action which could be inhibited by the peripheral CCK-A-receptor antagonist, devapezide (Figs 4-5). Devapezide also eliminated all spontaneous contractions in the absence of solution B, supporting the possibility of a CCK-liberation by the gallbladder itself.

In contrast, in the chicken experiment, solution B did not show any contractile effect on chicken gallbladder strips (Fig. 3). However it is not possible to determine whether this is because the chicken tissue had not secreted CCK, or whether the secretion was insufficient to stimulate gallbladder contraction.

The mammalian hormones gastrin and CCK share a common biologically active COOH-terminal pentapeptide sequence and have overlapping spectra of biological activities. However, there are important functional differences between the two peptides. CCK is a potent stimulant of gallbladder contraction and pancreatic enzyme secretion, while gastrin is largely ineffective in these systems, but it is a potent stimulation of gastric acid secretion (WALSH *et al.*, 1987). CCK-8 like molecules have previously been identified in avian brain and intestine (DOCKRAY, 1979), and a molecule identical to mammalian CCK-8 has been isolated from chicken brain (FAN *et al.*, 1987). The presence of an intestinal peptide that is both chemically and biologically similar to complete sequence mammalian CCK (1-33) in birds has not yet been proven.

DIMALINE *et al* (1990) have suggested that the factors which determine specificity of action of CCK and gastrin are different in birds and mammals. They have isolated chicken intestinal peptides which showed biological gastrin-like actions, but which were chemically more like CCK. This chicken CCK-like peptide was less potent than CCK-8 in causing contraction of both avian and mammalian gallbladder *in vitro*. A proline residue, immediately adjacent to the sulphated tyrosine in this chicken CCK-like peptide, may produce a steric effect that lowers its activity on gallbladder contraction (DIMALINE *et al.*, 1990). In our study, this steric effect could also explain the difference in effect between mammals and birds after stimulation with solution B.

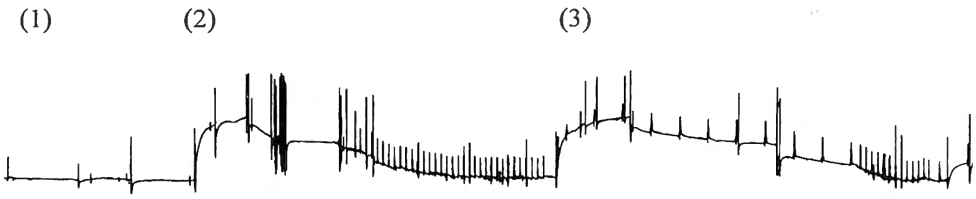

Fig. 1. — Simmondsin and CCK-8 were administered to mouse gallbladder with intermittent washes with KPS, in the following order : (1) 10 mM simmondsin, (2) 1 nM CCK-8/ml, (3) 1 nM CCK-8/ml.

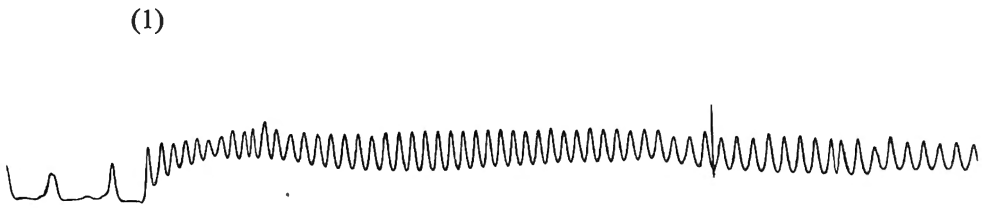

Fig. 2. — CCK-8 was administered to chicken gallbladder in a concentration of 100 nM CCK-8/ml (1).

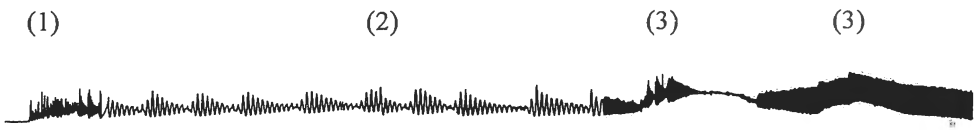

Fig. 3. — (1) Spontaneous contraction of chicken gallbladder begins. Chicken duodenal perfusate — after stimulation with 10 mM simmondsin - (solution B) (2) and 100 nM CCK-8/ml (3) were administered on chicken gallbladder tissue with intervening washes with KPS, in the following order : (2), (3), (3).

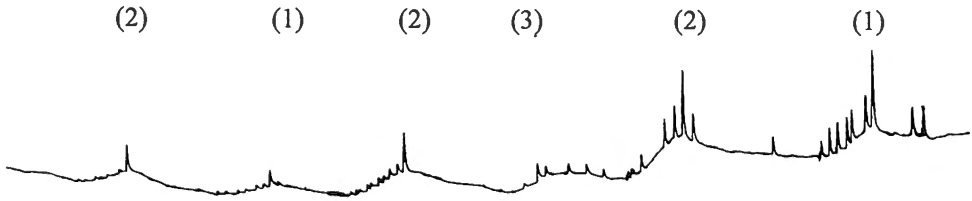

Fig. 4. — Guinea pig duodenal perfusate (1) before stimulation with simmondsin (solution A), or (2) after stimulation with 10 mM simmondsin (solution B) and (3) KPS were administered to guinea pig gallbladder tissue in the following order : (2), (1), (2), (3), (2), (1).

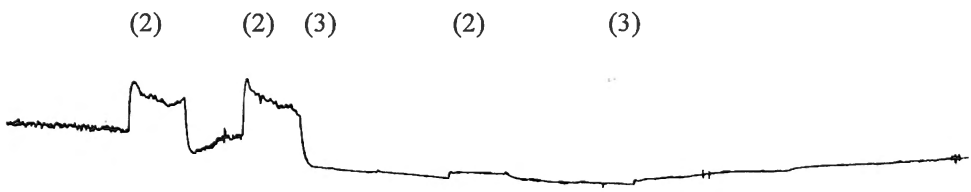

Fig. 5. — (2) Duodenal perfusate fluid after stimulation with 10 mM simmondsin (solution B) and (3) 1 µg/ml CCK-A antagonist were administered in the following order : (2), (2), (3), (2), (3).

In the *in vivo* studies, total gallbladder contraction was apparent by 15 minutes after i.p. injection of a high dose of (mammalian) CCK-8 (1 µg CCK-8/kg BW) in mice, as reported by others (MAKOVEC *et al.*, 1987). In contrast, no loss of gallbladder weight was observed in chickens, even at a higher dose of 5 nmol CCK-8/kg BW (Tables 1-2). The higher dose needed for *in vitro* gallbladder contraction both *in vitro* and *in vivo*, and the different pattern of contraction seen in chicken gallbladder, may explain why *in vivo* CCK did not empty gallbladders in chickens, whereas it did in mice.

From the *in vitro* studies it was already anticipated that an *in vivo* i.p. injection of a high dose of simmondsin (1 g/kg BW) would not elicite gallbladder contraction in chickens. It has not been proven yet that *in vivo*, simmondsin stimulates the liberation of CCK in mammals at a dose, effective to establish gallbladder contractions. However, it seems that endogenous CCK can stimulate gallbladder contractions in mice *in vivo*, because re-feeding the mice after a period of starvation caused a total gallbladder emptying (Table 1). The lack of effect of simmondsin in these studies may have been a result of looking at gallbladder weights using the standard 15 min test ; a longer period might have allowed time for simmondsin-stimulated, endogenous CCK production and gallbladder contraction to be seen. In birds, the presence and activity of chicken CCK is still not clear as gallbladders were not emptied after re-feeding . These results confirm the conclusions of MARTINEZ *et al.* (1993) that in birds the receptors mediating CCK-effects are different from those of mammals. The role of endogenous CCK in satiety in chickens has also been

questioned (COVOSA and FORBES, 1994) because an i.p. injection of devapezide could not block the reduction in feed intake after an i.p. injection of CCK-8.

TABLE 1

Mouse gallbladder weights 15 min. after i.p. injection of saline, CCK-8, simmondsin, PEG 400 + glycerol and saline, MK-329 + saline or after feed intake. Values are means ± standard errors (SE). Means with no common superscript differ significantly ($p < 0,05$) by Duncan's multiple range test

Treatment	*Gallbladder weight (mg) (mean ± SE) (n = 5)*
saline (ip)	25,4 ± 6,8[a]
CCK-8 (ip)	3,5 ± 0,7[b]
saline (ip)	25,4 ± 6,8[a]
simmondsin (ip)	40 ± 7,1[a]
PEG : glycerol (ip) + saline (ip)	58,7 ± 6,7[a]
devapezide (MK-329) (ip) + CCK-8 (ip)	30,8 ± 1,5[a]
after feed intake	6,7 ± 4,1[b]

TABLE 2

Chicken gallbladder weights 15 min or 30 min after i.p. injection of saline, CCK-8, simmondsin or after feed intake. Values are means ± standard errors (SE). Means with no common superscript differ significantly ($p < 0,05$) Duncan's multiple range test

Treatment	*Gallbladder weight after 15 min (mg) (mean ± SE) (n = 5)*	*Gallbladder weight after 30 min (mg) (mean ± SE) (n = 5)*
saline (ip)	96.8 ± 17.8[a]	107.4 ± 43.8[a]
CCK-8 (ip)	94.0 ± 11,5[a]	112.0 ± 10,2[a]
simmondsin (ip)	112.5 ± 8.6[a]	120.9 ± 9.9[a]
after feed intake	100.2 ± 15.4[a]	108.3 ± 9.3[a]

CONCLUSIONS

In conclusion, these *in vitro* studies show that.simmondsin does not directly stimulate CCK-receptors in mouse, chicken or guinea pig gallbladder. Our results suggest that simmondsin induces release of a CCK-like substance from guinea pig duodenum, but we could find no evidence that simmondsin could induce CCK production in chickens. *In vivo*, it could not be shown that simmondsin induced

gallbladder contraction in either mice or chickens ; it is even doubtful whether exogenous or endogenous CCK is able to stimulate chicken gallbladder contractions.

REFERENCES

ARNOUTS, S., J. BUYSE, M.M. COKELAERE and E. DECUYPERE (1993) — Jojoba meal (*Simmondsia chinensis*) in the diet of broiler breeder pullets : physiological and endocrinological effects. *Poultry Sci.*, **72** : 1714-1721.

BOOTH, A.N., C.A. ELLIGER and A.C. JR. WAISS (1974) — Isolation of a toxic factor from jojoba meal. *Life Sci.*, **15** : 1115-1120.

COKELAERE, M.M., H.D. DANGREAU, S. ARNOUTS, E.R. KÜHN and E. DECUYPERE (1992) — The influence of pure simmondsin on food intake in rats. *J. Agric. Food Chem.*, **40** : 1839-1842.

COKELAERE, M.M., P. DAENENS, G. FLO, E. KUHN, M. VAN BOVEN, S. VERMAUT, J. BUYSE and E. DECUYPERE — Simmondsin : physiological effects and working mechanisms in rats. *Proc. IX Intern. Conf. on jojoba and its uses*, Catamarca, Argentinia ; 25-29 September 1994, Ed. L.H. Princen ; A.O.C.S. 1995 (in press).

COVASA, M. and J.M. FORBES (1994) — Effects of the CCK receptor antagonist MK-329 on food intake in broiler chickens. *Pharmacol. Biochem. Behav.*, **48**(2) : 479-486.

DIMALINE, R. and C.M. LEE (1990) — Chicken gastrin : a member of the gastrin/CCK family with novel structure-activity relationships. *Am. J. Physiol.*, **259** : 882-G888.

DOCKRAY, G.J. (1979) — Cholecystokinin-like peptides in avian brain and gut. *Experientia*, **35** : 628-630.

ELLIGER, C.A., A.C. JR. WAISS and R.E. LUNDIN (1973) — Simmondsin, an unusual 2-cyanomethylenecyclohexyl glycoside from *simmondsia californica*. *J. Chem. Soc., Perkin Trans.*, **19** : 2209-2212.

ELLIGER C.A., A.C. JR. WAISS and R.E. LUNDIN (1974a) — Structure and stereochemistry of simmondsin. *J. Org. Chem.*, **39** : 2930-2931.

ELLIGER, C.A., A.C. JR. WAISS and R.E. LUNDIN (1974b) — Cyanomethylenecyclohexyl glycosides from *simmondsia californica*. *Phytochemistry*, **13** : 2319-2320.

FAN, Z.W., J. ENG, M. MIEDEL, J.D. HULMES, T.-C. E. PAN and R.S. YALOW (1987) — Cholecystokinin octapeptides purified from chinchilla and chicken brains. *Br. Res. Bull.*, **18** : 757-760.

MAKOVEC, F., M. BANI, R. CEREDA, R. CHISTA, M.A. PACINI, L. REVEL and L.C. ROVATI (1987) — Antispasmodic activity on the gallbladder of the mouse of CR 1409 (Lorglumide) a potent antagonist of peripheral CCK. *Pharmacol. Res. Communic.*, **19** : N° 1 : 41-48.

MANOS, C.G.JR., P.J. SCHRYNEMEECKERS, D.E. HOGUE, J.N. TELFORD, G.S. STOEWSAND, D.H. BEERMAN, J.G. BABISH ; J.T. BLUE ; B.S. SHANE and D.J. LISK (1986) — Toxicologic studies with lambs fed jojoba meal supplemented rations. *J. Agric. Chem.*, **34** : 801-805.

MARTINEZ V., M. JIMÉNEZ, GONALONS, P. VERGARA (1993) — Effects of cholecystokinin and gastrin on gastroduodenal motility and coordination in chickens. *Life Sci.*, **52** : 191-198.

NGOU NGOUPAYOU J.D.N., P.M. MAIORINO and B.L. REID (1982) — Jojoba meal in poultry diets. *Poult. Sci.*, **61** : 1692-1696.

SAS INSTITUTE (1985) SAS/STAT. — *Guide for Personal Computers. Version 6.* SAS Institute Inc., Cary, NC.

SHERBROOKE W.C. (1976) — Differential acceptance of toxic jojoba seed (*Simmondsia chinensis*) by four sonoran desert heteromyid rodents. *Ecology*, **57** : 596-602.

VAN BOVEN, M., N. BLATON, M.M. COKELAERE and P. DAENENS (1993) — Isolation, purification and stereochemistry of simmondsin. *J. Agric. Food Chem.*, **41** : 1605-1607.

WALSH, J.H. (1987) — Gastrointestinal hormones. *Physiology of the Gastrointestinal Tract.* L.R. Johnson (Ed.). New York, Raven : 181-252.