Lack of effect of the specific cholecystokinin receptor antagonist loxiglumide on cholecystokinin clearance from plasma in man

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Saline or loxiglumide (2.5 mg kg^{-1} in 10 min, followed by $5 \text{ mg kg}^{-1} \text{ h}^{-1}$ for 200 min) was administered intravenously in random order to six healthy subjects. After 60 min cholecystokinin (CCK-33) was infused ($0.5 \text{ i.d.u. kg}^{-1} \text{ h}^{-1}$ for 1 h then $1.0 \text{ i.d.u. kg}^{-1} \text{ h}^{-1}$ for 1 h). Loxiglumide did not change basal levels of CCK and did not augment plasma CCK-immunoreactivity during CCK-33 infusion. After cessation of the CCK-infusion, plasma CCK concentrations decreased rapidly to basal values within 12 min, and the elimination half-life during loxiglumide infusion ($4.8 \pm 0.8 \text{ min}$) was not significantly different from that during saline infusion ($4.2 \pm 1.0 \text{ min}$). These results suggest that loxiglumide does not interfere with the distribution and metabolism of CCK.

Keywords CCK receptor antagonists loxiglumide cholecystokinin elimination half-life

Introduction

In previous studies we have shown that plasma cholecystokinin (CCK) concentrations in response to a meal (Jansen *et al.*, 1989a; Meyer *et al.*, 1989) or to intravenously administered bombesin (Jansen *et al.*, 1989a) are augmented by the specific CCK-receptor antagonist loxiglumide (Setnikar *et al.*, 1988) in man. Furthermore, gallbladder contraction in response to these stimuli was virtually abolished by loxiglumide (Douglas *et al.*, 1989; Meyer *et al.*, 1989), supporting an important role for the gallbladder in a negative feedback mechanism between bile-products in the lumen of the proximal small intestine and CCK-release (Gomez *et al.*, 1988; Koop *et al.*, 1988, 1989).

However, increased plasma CCK-concentrations found during loxiglumide administration in these studies may also be explained by other mechanisms, such as an acceleration of gastric emptying of nutrients into the small intestine induced by CCK-receptor antagonism (Meyer *et al.*, 1989) or impaired clearance of CCK by these receptor antagonists (Hosotani *et al.*,

1987). The latter possibility was investigated in the present study.

Methods

Six healthy subjects (3F, 3M; mean age $\pm$ s.d.: 51 ± 11 years) were studied on two occasions separated by at least 2 weeks. The order of the two experiments was randomized.

After an overnight fast (of at least 12 h) the volunteers presented at the laboratory, where two indwelling intravenous catheters were placed one in each forearm. The catheters were kept patent by a saline infusion. One catheter was used for the collection of blood samples, while saline or loxiglumide (CR-1505; Rotta laboratories, Monza, Italy: $2.5 \text{ mg kg}^{-1} 10 \text{ min}^{-1}$ followed by $5.0 \text{ mg kg}^{-1} \text{ h}^{-1}$ for 200 min) was infused through the other. Sixty minutes after the start of the saline or loxiglumide infusion cholecystokinin (porcine CCK-33; Ferring AB, Malmö, Sweden) was infused intravenously in a

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dose of 0.5 i.d.u. CCK $\text{kg}^{-1} \text{h}^{-1}$ for 60 min, followed by 1.0 i.d.u. CCK $\text{kg}^{-1} \text{h}^{-1}$ for another 60 min. The first infusion rate was designed to simulate plasma CCK concentrations achieved after the stimulus of a meal (Jansen *et al.*, 1989b), and the second infusion rate resulted in plasma CCK levels comparable with those obtained after infusion of 2.5 ng $\text{kg}^{-1} \text{min}^{-1}$ bombesin (Douglas *et al.*, 1989). Blood samples were collected at 15 min intervals for the first 180 min then at 1, 2, 4, 6, 8, 10, 12, 15, 20, 25, 30, 40, 50 and 60 min after the end of CCK administration.

The samples were collected on ice in EDTA-containing glass tubes and were centrifuged immediately at 4°C. Plasma was stored at -20°C until assayed for CCK. The concentrations of cholecystokinin were measured by radioimmunoassay as described by Jansen & Lamers (1983a,b). The CCK antiserum T204 reacts fully with CCK-39, CCK-33 and CCK-8s and only binds to negligible amounts of sulphated gastrins (<2%), while no crossreactivity occurs with unsulphated molecular forms of gastrin, CCK-8 and structurally unrelated peptides. The detection limit was below 1.0 pM. A 50% inhibition of maximum binding was produced at 2.8 pM. The intra-assay variance ranged from 4.6 to 11.5% and the inter-assay variance from 11.3 to 26.1% in the working range of concentrations.

Statistical analysis

Plasma CCK concentration-time data were expressed as mean $\pm$ s.d. The elimination half-life of plasma CCK after cessation of CCK-infusion was calculated as described by Walsh *et al.* (1974).

Student's *t*-test for paired values was used to assess differences between means. Differences with a *P* value of <0.05 were considered to be significant.

The studies were approved by the local ethics committee and all subjects gave informed consent.

Results

Mean basal plasma CCK levels during saline infusion ($3.7 \pm 1.2 \text{ pmol l}^{-1}$) were not significantly influenced by loxiglumide ($4.0 \pm 1.5 \text{ pmol l}^{-1}$).

CCK concentrations measured during the two rates of CCK-33 infusion were also not influenced significantly by loxiglumide (Figure 1).

After cessation of the CCK infusion, plasma CCK concentrations declined rapidly and there was no statistically significant difference in the elimination half-life regardless of whether loxiglumide or saline was infused (Table 1).

Figure 1 Effect of loxiglumide (○) and saline (●) on plasma CCK concentrations before, during and after an i.v. infusion of CCK-33 at two different rates (0.5 and 1.0 i.d.u. $\text{kg}^{-1} \text{h}^{-1}$) in six healthy subjects. Results are expressed as mean $\pm$ s.e. mean.

Table 1 Individual elimination half-lives of CCK after cessation of the CCK infusion, with and without infusion of loxiglumide (CR-1505) in six healthy subjects. Values were calculated from elimination constants obtained by linear regression of the logarithm of concentration vs time.

Patient	$t_{1/2}$ (min)	
	Saline	Loxiglumide
1	4.3	5.6
2	3.2	4.8
3	4.2	3.9
4	4.0	4.5
5	6.1	6.0
6	3.5	4.1

Discussion

The results suggest that increased plasma CCK concentrations observed after meal stimulation and during bombesin infusion are not the result of impaired metabolism of CCK by loxiglumide.

It has previously been shown that circulating peptides like CCK are rapidly metabolised in capillaries throughout the body (Strunz *et al.*, 1978), especially in the kidney (Thompson *et al.*, 1975). However, small molecular forms of CCK

are also extracted effectively by the liver (Sakamoto *et al.*, 1985). It might therefore be argued that molecular forms of CCK other than the one infused are metabolised differently during loxiglumide administration. This hypothesis, however, is unlikely since plasma CCK levels during CCK-8s infusion are not augmented by CCK-receptor antagonists (Cantor *et al.*, 1989; Schmidt *et al.*, 1989), and since plasma levels of endogenously released CCK during bombesin infusion resulting in circulating molecular forms (Jansen & Lamers, 1983c), comparable with those released after a meal (Jansen & Lamers, 1987), also rapidly declined after cessation of the bombesin infusion, despite continuous administration of loxiglumide (Jansen *et al.*, 1989a).

This study further supports the existence of a negative feedback mechanism between plasma CCK and bile products in the lumen of the proximal small intestine (Adler *et al.*, 1988; Gomez *et al.*, 1988; Koop *et al.*, 1988, 1989). However, other mechanisms of an augmented stimulated CCK release during loxiglumide administration, such as direct effects of loxiglumide on CCK-producing cells or indirect effects via yet to be established paracrine, neurocrine or endocrine mechanisms cannot be excluded.

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