# Oxyntomodulin (glucagon-37) and its C-terminal octapeptide inhibit gastric acid secretion

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Oxyntomodulin (OXM) is a peptide isolated from porcine intestine which consists of the whole glucagon sequence with a basic octapeptide ($KA_8$) at its C-terminal end. In this study, the effect of OXM and $KA_8$ on pentagastrin-stimulated gastric acid secretion has been studied in conscious rats and cats. In rats, OXM (25–450 pmol · $kg^{-1}$) as well as $KA_8$ (7.5–60 nmol · $kg^{-1}$) inhibited pentagastrin-stimulated gastric acid output in a dose-dependent manner; $KA_8$ was about 100-times less potent than OXM. In cats, $KA_8$ (90 nmol · $kg^{-1}$) was also an inhibitor of acid secretion. We conclude that OXM, or a closely related peptide, could be a physiological modulator of gastric acid secretion, and that the C-terminal octapeptide of OXM is implicated in this effect.

*Oxyntomodulin* *Rat* *Cat* *Acid secretion* *Pentagastrin*

## 1. INTRODUCTION

Oxyntomodulin [1] is a peptide isolated from porcine jejuno-ileum, which consists of the whole glucagon sequence with a basic octapeptide at its C-terminal end [2]. The comparison between the biological activities of glucagon and oxyntomodulin has shown that oxyntomodulin (OXM) (25–250 pmol · $kg^{-1}$) is 10–20-times more potent than glucagon in inhibiting pentagastrin (PG)-stimulated gastric acid secretion in the anaesthetised rat [3]. This observation suggests that OXM may be a physiological modulator of gastric acid secretion and that the C-terminal octapeptide ($KA_8$) which differentiates OXM from glucagon may be implicated in the biological activity of OXM.

To examine these points, $KA_8$ has been synthesized, and both OXM and $KA_8$ have been examined for their ability to interfere with PG-stimulated acid secretion in conscious rats, an animal whose secretion is under vagal and splanchnic control. The effect of $KA_8$ on acid secretion has also been studied in the cat. It has been found that both OXM and $KA_8$ inhibit PG-stimulated acid secretion, OXM being about a 100-times more potent than $KA_8$ in the rat.

## 2. MATERIALS AND METHODS

### 2.1. *Synthesis of the octapeptide*

The octapeptide, $KA_8$ (Lys-Arg-Asn-Lys-Asn-Asn-Ile-Ala-OH), was synthesized by stepwise acylations in 'liquid phase' with active esters. The methods of synthesis used have been described in detail [4]. The benzyloxycarbonyl group was used for the protection of the $N_\alpha$ amino group, the *tert*-butyloxycarbonyl group for protection of the side chains of lysine, and the *tert*-butyl ester for protection of the C-terminal alanine. Arginine was introduced as its tribenzyloxycarbonyl derivative and then left unprotected. The last lysine was coupled as its bisterbutyloxycarbonyl active ester derivative. Final deprotection of the peptide was

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performed with trifluoroacetic acid. The purity of the complete unprotected peptide was checked by high-performance liquid chromatography (μBondapak C18 column). The octapeptide was identified by elemental analysis, amino acid analysis and by its $^1$H NMR spectrum (360 MHz).

### 2.2. *Experiments in conscious rats*

Wistar rats (IFFA CREDO, France) (300 g) provided with a chronic gastric fistula [5], were conditioned to stay 5 h in Bollman-type cages. Tests began 14 days after implantation of the cannula. The animals were starved for 18 h before the experiment with free access to tap water. The gastric juice was collected by gravity-drainage through the fistula over 20-min periods. Fractions were weighed and analysed for acid concentration by titration with 0.01 N NaOH using 1% phenolphthalein as an indicator. Acid output was calculated by multiplying the volume of secretion by the acid concentration. Saline and pentagastrin (Peptavlon, ICI) ($0.5\ \mu g \cdot kg^{-1} \cdot h^{-1}$) were administered as intravenous infusions at a rate of 2 ml/h through silastic (Dow Corning) tubing. OXM and $KA_8$ were dissolved in a sterile physiological saline solution containing bovine serum albumin (fraction V, Sigma, 0.5%) and injected as a bolus in a 400 μl volume. Natural oxyntomodulin [1] extracted from pig intestine was used. It contained no contaminants when analysed by high-performance liquid chromatography. The statistical probabilities were calculated by the Student's *t*-test.

### 2.3. *Experiments in conscious cats*

Three adult cats (body wt 3.5–4.5 kg) were provided with a gastric fistula 12–18 months before these experiments. Before each test, food, but not water, was withheld for 18 h. The interval between tests was at least 1 week, all tests were done in a random order. Throughout the experiments, saline was infused intravenously at a rate of 12 ml/h. The gastric juice was collected in 15-min periods and acid output was calculated from determinations of HCl concentration (titration end point 7.8) and volumes. Basal secretion was studied for at least 30 min and stimulated secretion for 3 h. $KA_8$ was given as a bolus injection on the plateau PG secretion. Statistical probabilities were calculated by the Student's *t*-test.

## 3. RESULTS

### 3.1. *Experiments in conscious rats*

Under basal conditions, the volume of gastric juice collected during 20 min was 507 ± 77 μl, the acid concentration was 70 ± 9 meq/l, corresponding to an acid output of 37 ± 5 μeq/20 min ($n$ = 7). Under stimulation by PG ($0.5\ \mu g \cdot kg^{-1} \cdot h^{-1}$), the volume and the acid concentration doubled and the resulting acid output tripled. The PG effect corresponded to 65–70% of the maximal acid secretory response.

Natural porcine OXM (225 pmol/kg), when bolus injected, decreased both the volume of the gastric juice and the acid concentration, resulting in a decrease in the output of acid (fig.1). Inhibition of PG-stimulated acid output attained about 40% and was observed over 3 consecutive 20-min collection periods. Comparable effects were observed with synthetic $KA_8$ (fig.2), albeit with a higher dose (30 nmol/kg).

The acid secretion induced by PG during 120 min was calculated; the inhibition of this

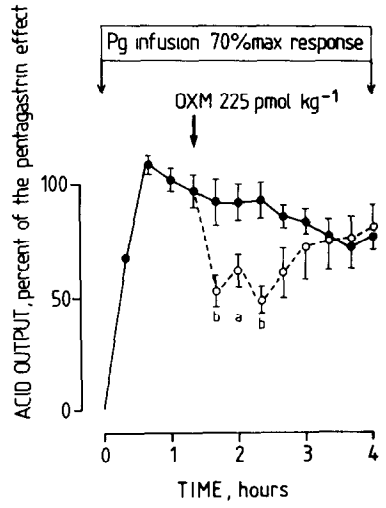


Fig.1. Effect of a bolus injection of OXM on PG-stimulated gastric acid secretion in conscious rats provided with chronic gastric fistula. Data from OXM-injected rats (○) are expressed relatively to the plateau of stimulation obtained with perfusion of PG alone (●). Basal values (without PG) were deduced from each experimental point. Results are given as mean ± SE, $n$ = 7. The statistical significance was performed against the control obtained at the same time. (a) $p < 0.05$, (b) $p < 0.002$.

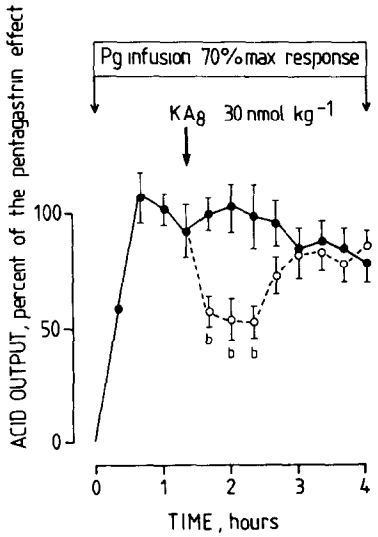


Fig.2. Effect of the C-terminal octapeptide ($KA_8$) of porcine OXM on the PG-stimulated acid output in conscious rat. PG (●), mean ± SE of 7 experiments; PG plus $KA_8$ (○), mean ± SE of 7 experiments; (b) $p <$ 0.002.

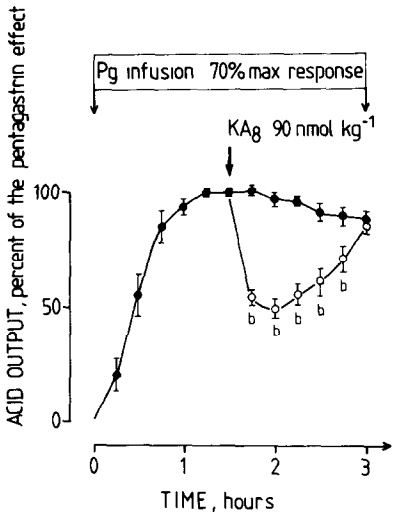


Fig.4. Effect of the C-terminal octapeptide ($KA_8$) of porcine OXM on PG-stimulated acid output in conscious cat. PG (●), mean ± SE of 6 experiments; PG plus $KA_8$ (○), mean ± SE of 6 experiments; (b) $p <$ 0.002.

secretion as a function of increasing doses of OXM and $KA_8$ is shown in fig.3. $KA_8$ was 133-times less potent than OXM in inhibiting gastric acid secretion.

### 3.2. *Experiments in conscious cats*

When the acid secretion of conscious cats was submaximally stimulated by PG (70% of the maximal response), the synthetic octapeptide $KA_8$ (90 nmol/kg) inhibited gastric acid secretion: 45–50% inhibition was attained over 3 consecutive 15-min periods (fig.4).

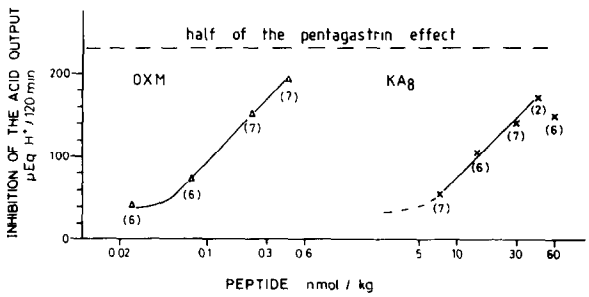


Fig.3. Effect of OXM or its C-terminal octapeptide ($KA_8$) on PG ($0.5\ \mu g \cdot kg^{-1} \cdot h^{-1}$) stimulated acid output as a function of the dose in conscious rat. Number of experiments in parentheses.

## 4. DISCUSSION

The jejuno-ileum and the endocrine pancreas of the rat contain large quantities of OXM [6]. In the rat, the presence of OXM in plasma was clearly shown [6]. OXM or molecules derived therefrom could be released under physiological circumstances and regulate gastric functions. Our results confirm that OXM inhibits PG-stimulated gastric acid secretion and indicate that the vagal tone is compatible with this inhibitory effect. Furthermore, they indicate that $KA_8$, the synthetic replica of the C-terminal octapeptide that differentiates OXM from glucagon can also inhibit acid secretion although being 100–150-times less potent than OXM. This is also the case in the cat. Recently, molecular cloning techniques have allowed the characterization of the OXM sequence in rat and man [7,8]. In these species, an arginine residue replaces the lysine present in position 4 of porcine $KA_8$ (position 33 in OXM). The sequence of cat OXM is unknown. According to our data, it would appear that the structural differences between the OXM of mammals have little functional importance, if any.

As far as the mode of action of OXM and $KA_8$ is concerned, OXM is 10–20-fold less potent than

glucagon in its interaction with glucagon receptors in liver [2], adipose tissue (unpublished) and heart. In contrast, a receptor which preferentially binds OXM has been described in the rat fundic glands [9]; these glands also display a cyclic AMP system which is more sensitive to OXM than to glucagon [10]. The similar specificities exhibited by the OXM binding sites [9], the oxyntic glands cyclic AMP system [10] and the inhibition of gastric acid secretion, suggest that these functional entities are related. However, the precise biochemical events which link the binding sites with the biological activity and the role of cyclic AMP in this process remain unknown.

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