Bioorganic & Medicinal Chemistry Letters, Vol.3, No.5, pp. 851-854, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

ON THE SIGNIFICANCE OF THE C-TERMINAL PRIMARY AMIDE IN CHOLECYSTOKININ

Marc Rolland, Marie-Françoise Lignon, Marie-Christine Galas, Nicole Bernad, Marc Rodriguez, Pierre Fulcrand and Jean Martinez*

CCIPE-Faculté de Pharmacie, 15 Av. C. Flahault, 34060 Montpellier, Cédex, France.

(*Received* 13 *July* 1992; *accepted* 2 *October* 1992)

Abstract : We report on the synthesis of some analogs of the C-terminal heptapeptide of cholecystokinin in which the C-terminal primary amide has been deleted and the L-tryptophan replaced by a D-tryptophan. These analogs were evaluated *in vitro* for their ability (i) to recognize the CCK receptor on rat pancreatic acini and on guinea pig brain membranes; (ii) to stimulate amylase release from rat pancreatic acini. Although previous works suggested that the C-terminal primary amide is essential for exhibiting a full biological response on pancreatic acini, this study does not confirm this hypothesis.

Cholecystokinin (CCK) is a hormone and proposed neurotransmitter found in gut and brain. It interacts with nanomolar affinities with at least two types of binding sites designated CCK-A (peripheral) and CCK-B (central) receptors. Two different classes of CCK-A binding sites with high and low affinity for CCK have been defined (1). It has been proposed that the occupancy of high-affinity binding sites correlates with stimulation of amylase secretion and that occupancy of low-affinity binding sites correlates with inhibition of amylase secretion (2). Intracellular events and biological activities associated with high- and low-affinity binding sites have been identified (3, 4).

We have developed a series of CCK-analogs in which the C-terminal primary amide function has been deleted, such as compounds **JMV180**, Boc-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-2-Phenylethylester, and Boc-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-2-Phenylethyl amide **1** (2). We and others demonstrated that this class of compounds act as agonists at the high-affinity binding sites and as antagonists at the low-affinity binding sites (2, 5, 6). In such compounds which lack the C-terminal primary amide, replacing the tryptophan by a D-tryptophan resulted in full CCK-receptor antagonists (7). All these findings demonstrate the functional role of the C-terminal carboxamide moiety of CCK and illustrate the significance of the tryptophan residue for CCK peripheral biological activity.

The objective of the present study is to examine in more detail the effects of the C-terminal amide suppression combined with C-terminal side-chain modifications and L-tryptophan substitution by a D-tryptophan. The resulting analogs (Figure 1) were evaluated for their ability to

interact with CCK-A binding sites in rat pancreatic acini and with CCK-B binding sites on guinea pig brain membranes, as well as for their ability to stimulate amylase secretion from rat pancreatic acini. They were compared with the potent CCK analog Boc(Nle^{28,31})CCK-$_{27-33}$ (8, 9) and with compounds **1** and **2**. The detailed syntheses and characterization of these analogs will be described in a separate report.

Figure 1 : Chemical structures of the CCK-analogs. All compounds were purified by HPLC and were identified by ^{1}H NMR spectroscopy, mass spectrometry and aminoacid analysis.

Boc-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-Phe-NH_2, Boc(Nle^{28,31})CCK-$_{27-33}$, (**JMV236**)
Boc-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-NH-$(CH_2)_2$-C_6H_5 (**1**)
Boc-Tyr(SO_3H)-Nle-Gly-DTrp-Nle-Asp-NH-$(CH_2)_2$-C_6H_5 (**2**)
Boc-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-NH-CH_2-CH-$(C_6H_5)_2$ (**3**)
Boc-Tyr(SO_3H)-Nle-Gly-DTrp-Nle-Asp-NH-CH_2-CH-$(C_6H_5)_2$ (**4**)
Boc-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-NH-CH-$(CH_2$-$C_6H_5)_2$ (**5**)
Boc-Tyr(SO_3H)-Nle-Gly-DTrp-Nle-Asp-NH-CH-$(CH_2$-$C_6H_5)_2$ (**6**)
Boc-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-N(CH_3)-$(CH_2)_2$-C_6H_5 (**7**)
Boc-Tyr(SO_3H)-Nle-Gly-DTrp-Nle-Asp-N(CH_3)-$(CH_2)_2$-C_6H_5 (**8**)

As seen in Table 1, the analogs were able to recognize the CCK receptors with different affinities and potencies. As previously reported, compound **1** with no C-terminal amide function exhibits the maximal response of amylase secretion from rat pancreatic acini with a plateau of stimulation. However, replacement of the L-tryptophan by a D-tryptophan in such an analog results in producing a CCK-receptor antagonist (e. g. compound **2**) with an IC_{50} of about 30μM, confirming the significance of both the C-terminal amide function and the orientation of the indole side chain of tryptophan for full biological activity at both high- and low-affinity CCK receptors. Similar results were obtained with compound **7**, an analog of compound **1** in which the nitrogen of the 2-phenylethylamide has been methylated. Compound **7** fully stimulated amylase secretion with a plateau of stimulation at high concentrations. Compound **7** has about twice the affinity of compound **1** in recognizing the CCK receptor on rat pancreatic acini. Again, replacing the L-tryptophan by a D-tryptophan in compound **7** led to a full CCK-receptor antagonist (e. g. compound **8**) with an IC_{50} of about 26μM (Table 1). Surprisingly, although

compounds **3** and **5** do not possess the C-terminal amide function, they exhibited the same biphasic dose-response curve as CCK-8 or its potent analog Boc(Nle^{28,31})CCK-$_{27-33}$. They fully stimulate amylase release from rat pancreatic acini with a decrease of the maximal response at high concentrations. In these analogs (compounds **4** and **6**), replacement of the L-tryptophan by D-tryptophan did not yield CCK antagonists but produced CCK agonists with full efficacy and decreased potency.

The results of this paper also confirm previous observations that, any modification affecting the C-terminal tetrapeptide part of the CCK molecule, resulted in decreased potency at the CCK-B receptor type (Table 1).

Table 1. Biological activities of the CCK-analogs, on the binding to Rat pancreatic acini and Guinea Pig brain membranes and on amylase secretion from rat pancreatic acini.

CCK-analogs	Rat Pancreatic Acini		Guinea Pig Brain membranes
	Amylase Secretion	Binding	Binding
	EC_{50} (μM)	IC_{50} (μM)	IC_{50} (nM)	IC_{50} (nM)
JMV236	0.00005 ± 0.00001	-	2.3 ± 0.5	0.29 ± 0.05
1	0.030 (p 100%)	-	100	10
2	-	antagonist 30	500	1000
3	0.032 ± 0.03	-	700 ± 100	53 ± 5
4	9.3 ± 4	-	2200 ± 500	600 ± 150
5	0.70 ± 0.16	-	6200 ± 700	72 ± 25
6	86 ± 12	-	12000 ± 1200	700 ± 200
7	0.07 ± 0.03 (p 100 %)	-	48 ± 6	80 ± 17
8	-	antagonist 26 ± 5	1100 ± 250	1800 ± 400

(p) : plateau of stimulation. In each experiment, each value was determined in duplicate and the results are means from at least three separate experiments. Pancreatic acini (10, 11) and brain membranes (12) were prepared as previously described. Amylase release was measured using the procedure already described (10, 11). Apparent affinities of the tested analogs (IC_{50}'s) were determined by inhibition of binding of labeled CCK-8 (^{125}I-BH-CCK-8) as previously described (10, 11, 12).

In this communication, we have demonstrated that in contrast with previously reported results, the C-terminal amide function of cholecystokinin is not essential for full agonist activity at both the high and low-affinity CCK binding sites. It is interesting to note that in CCK-analogs that behave as agonists at the high-affinity binding sites and as antagonists at the low-affinity binding sites, e. g. compounds **1**, **7** showing a plateau of stimulation on amylase secretion in rat pancreatic acini, replacement of the L-tryptophan by a D-tryptophan produces full CCK-antagonists. On the other hand, replacement of the L-tryptophan by a D-tryptophan in full CCK agonists (e. g. compounds **3** and **5**) led to full CCK agonists with decreased potency. In terms of structure-activity relationships, these results have significance, since high- and low-affinity CCK binding sites are differently involved in the control of biological activities of CCK (13).

References

1. Yu, D. H., Huang, S. C., Wank, S. A., Mantey, S., Gardner, J. D. and Jensen, R. T. *Am. J. Physiol.*, **1990**, 258, G86-G95.
2. Galas, M. C., Lignon, M. F., Rodriguez, M., Mendre, C., Fulcrand, P., Laur, J., and Martinez, J. *Am. J. Physiol.*, **1988**, 254, G176-G182.
3. Matozaki, T., Göke, B., Tsunoda, Y., Rodriguez, M., Martinez, J., and Williams, J.A. *J. Biol. Chem.*, **1990**, 265, 6247-6254.
4. Lignon, M. F., Galas, M. C., Rodriguez, M., and Martinez, J. *Cell Sign.*, **1990**, 2, 339-346.
5. Stark, H. A., Sharp, C. M., Sutliff, V. E., Martinez, J., Jensen, R. T. ,and Gardner, J. D. *Biochim. Biophys. Acta*, **1989**, 1010, 145-150.
6. Matozaki, T., Martinez, J., and Williams, J. A. *Am. J. Physiol.*, **1989**, 257, G594-G600.
7. Lignon, M. F., Galas, M. C., Rodriguez, M., Laur, J., Aumelas, A., and Martinez, J. *J. Biol. Chem.*, **1987**, 262, 7226-7231.
8. Ruiz-Gayo, M., Daugé, V., Menant, I., Begué, D., Gacel, G., and Roques, B. P. *Peptides*, **1985**, 6, 415-420.
9. Rodriguez, M., Lignon, M. F., Galas, M. C., Fulcrand, P., Mendre, C., Aumelas, A., Laur, J., and Martinez, J., *J. Med. Chem.*, **1987**, 30, 1366-1373.
10. Jensen, R. T., Lemp, G. F., and Gardner, J. D. *J. Biol. Chem.*, **1982**, 257, 5554-5559.
11. Sankaran, H., Golfine, I. D., Bailey, A., Licko, V., and Williams, J. A. *Am. J. Physiol.*, **1982**, 245, G250-G257.
12. Pelaprat, D., Zajac, J. M., Gacel, G., Durieux, C., Morgat, J. L., Sasaki, A., and Roques, B. P. *Life Sci.*, **1985**, 37, 2483-2490.
13. Martinez, J., Galas, M. C., Lignon, M. F., Rodriguez, M., Fulcrand, P. **1991**, In : *Cholecystokinin Antagonists in Gastroenterology*, Adler, G. and Beglinger C. Eds, Springer Verlag, Berlin, Heidelberg, 80-90.