Vol. 152, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1988 Pages 203-207

BIOLOGICALLY ACTIVE CALCITONIN ANALOGS WHICH HAVE MINIMAL INTERACTIONS WITH PHOSPHOLIPIDS

Richard M. Epand[1], Raquel F. Epand[1] and Ronald C. Orlowski[2]

[1]Department of Biochemistry, McMaster University Health Sciences Centre, Hamilton, Ontario, Canada, L8N 3Z5

[2]Armour Pharmaceutical Company, Kankakee, Illinois, 60901

Received March 4, 1988

A number of peptide hormones have been shown to contain amphipathic helical segments capable of binding to phospholipids. This conformational feature has been associated with increased biological activity of these hormones. We demonstrate, however, that two calcitonin analogs, [Gly^8,Ala^{16}]-des-Leu^{19} salmon calcitonin and des-1-amino-[$Ala^{1,7}$,Gly^8]-des-Leu^{19} salmon calcitonin have minimal interactions with phospholipids. Neither of these peptides acquire any increased helical content in the presence of dimyristoylphosphatidylglycerol and these peptides have only weak effects in altering the phase transition properties of this lipid. Therefore, although the presence of a phospholipid-induced amphipathic helical sequence may enhance the biological activity, it is not required for activity.

Ever since the first demonstration of the binding of an amphipathic helix-forming peptide hormone with lipid (1) there has been a growing interest in the role of this conformational feature in membrane active peptides. Kaiser and his coworkers have dramatically demonstrated that residues in amphipathic helix-forming regions of peptide hormones can be replaced without loss of biological activity (2). However, other factors such as conformational flexibility and long range interactions, in addition to specific effects, also modulate hormone potency (3,4). In this work, we demonstrate that analogs of salmon calcitonin (sCT) that have almost completely lost their ability to interact with lipid still retain biological activity.

MATERIALS AND METHODS

The conformational properties of the calcitonin analogues were studied with circular dichroism (CD). The CD of peptides and proteins is indicative of their secondary structure. The mean residue ellipticity at 222 nm becomes more negative as a result of increased secondary structure content, particularly helical structures.

Details of the peptide syntheses will be reported elsewhere; experimental protocols for the CD and differential scanning calorimetry (DSC) experiments are as previously published (5,6).

0006-291X/88 $1.50

RESULTS

Calcitonin and its analogs exhibit little secondary structure in aqueous solution (Table I). In methanol solution, all of the analogs exhibit increased helical content as they do in the presence of the detergents lysolecithin (LPC) or sodium dodecyl sulfate (SDS) (Table I). However, a more critical test of the ability of a peptide to bind to lipid is an increased helical content in the presence of phospholipids. Two of the analogs listed in Table I, [Gly^8,Ala^{16}]-des-Leu^{19}-sCT and des-1-amino-[$Ala^{1,7}$,Gly^8]-des-Leu^{19}-sCT show no increase in helical content in the presence of dimyristoylphosphatidylglycerol (DMPG). The slight decrease in the magnitude of the 222 nm ellipticity for these two samples in the presence of DMPG may be due to scattering artifacts. These were the only two peptides that did not completely clarify turbid multilamellar suspensions of DMPG.

The interaction of these peptides with DMPG was also studied by DSC (Fig. 1). The pure lipid exhibits a cooperative phase transition at 22.3° with an enthalpy of 5.7 Kcal/mol. There is a premelt transition at 11.3° with an enthalpy of 0.6 Kcal/mol. Addition of sCT or [Gly^8]-sCT at a 10:1 lipid to peptide molar ratio greatly broadens the main transition and eliminates the pre-transition. The enthalpy of the main transition is reduced to 4.0 Kcal/mol DMPG with [Gly^8]-sCT and to 3.3 Kcal/mol DMPG with sCT. The only other peptide used in this study which reduced the main transition enthalpy of DMPG is des-1-amino-[$Ala^{1,7}$]-des-Leu^{19}-sCT which reduced the transition enthalpy to 5.0 Kcal/mol

Table 1. CD and *In Vivo* Hypocalcemic Activity of sCT and Analogs

	$-[\theta]_{222}$ (deg cm^2 $dmol^{-1}$)					
Peptide	No Additions	DMPG	LPC	SDS	Methanol	Hypocalcemic Activity (IU/mg)
sCT	4,160	12,350	10,830	11,410	16,065	4,250
[Gly^8]-sCT	2,915	5,400	5,325	7,925	N.D.	6,500
[Gly^8]-des-Leu^{19}-sCT	3,600	4,230	4,750	9,630	13,790	11,000
[Gly^8,Ala^{16}]-des-Leu^{19}-sCT	3,300	2,170	4,670	7,610	13,450	2,500
Des-1-Amino-[$Ala^{1,7}$]-Leu^{19}-sCT	2,740	5,350	6,010	11,040	16,850	7,400
Des-1-Amino-[$Ala^{1,7}$, Gly^8]-des-Leu^{19}-sCT	2,260	1,990	3,370	6,970	13,910	5,400

100 μM peptides in the presence or absence of 1 mM DMPG, 2.5 mM LPC or 25 mM SDS at 25° in 20 mM PIPES, 1 mM EDTA, 0.15 M NaCl, 0.02 mg/mL NaN_3, pH 7.40

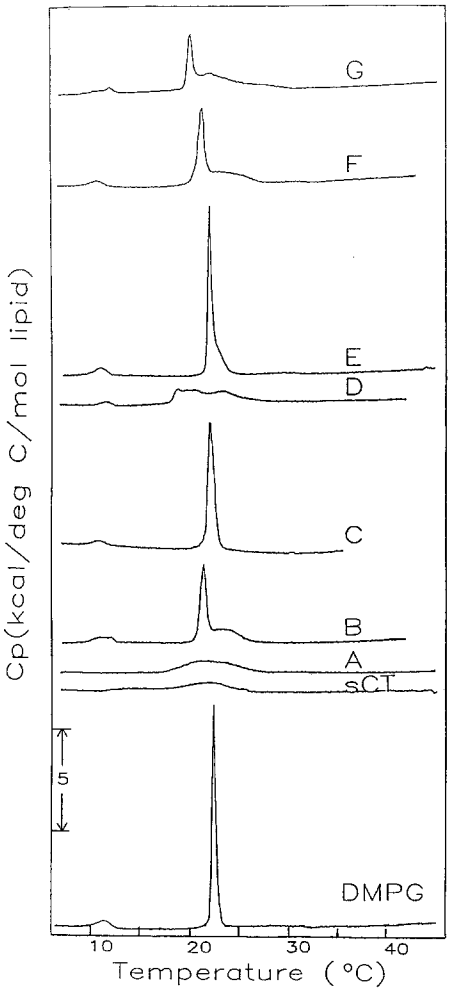

Figure 1. DSC scans of DMPG alone and in the presence of peptides. All samples contain 1 mM DMPG in 20 mM PIPES, 150 mM NaCl, 1 mM EDTA, 0.02 mg/mL NaN_3, pH 7.4. Heating scan rate 0.7 K/min. Calibration mark, 5 Kcal/deg K/mol lipid. DMPG, pure lipid and DMPG containing: sCT, 100 μM sCT; A, 100 μM [Gly^8]-sCT; B, 100 μM [Gly^8]-des-Leu^{19}-sCT; C, 100 μM [Gly^8,Ala^{16}]-des-Leu^{19}-sCT; D, 100 μM des-1-amino-[$Ala^{1,7}$]-des-Leu^{19}-sCT; E, 100 μM des-1-amino-[$Ala^{1,7}$,Gly^8]-des-Leu^{19}-sCT; F, 1 mM [Gly^8,Ala^{16}]-des-Leu^{19}-sCT; G, 1 mM des-1-amino-[$Ala^{1,7}$,Gly^8]-des-Leu^{19}-sCT.

lipid (Fig. 1, curve D). At 10:1 DMPG/peptide molar ratio both [Gly^8,Ala^{16}]-des-Leu^{19}-sCT and des-1-amino-[$Ala^{1,7}$,Gly^8]-des-Leu^{19}-sCT only slightly broaden the transition and have no effect on the transition enthalpy nor do they abolish the pre-transition (Fig. 1, curves C and E). Only at a very low DMPG/peptide molar ratio of 1 does the broadening of the transition become significant with these peptides (Fig. 1, curves F and G).

DISCUSSION

It is clear that [Gly^8,Ala^{16}]-des-Leu^{19}-sCT and des-1-amino-[$Ala^{1,7}$,Gly^8]-des-Leu^{19}-sCT have very little interaction with DMPG. They do not undergo a

conformational change in the presence of this lipid and they have almost no effect on the phase transition properties of this lipid at a 10:1 lipid to peptide molar ratio. Nevertheless, these peptides still have a weak affinity for amphiphiles. They can acquire a higher helical content in the presence of detergents or organic solvent (Table 1) and they can affect the phase transition properties of DMPG at high concentrations (Fig. 1). These properties, however, are not very specific and all calcitonin analogs we have thus far tested, both active and nonactive, show some increase in the magnitude of $[\theta]_{222}$ in LPC, SDS and methanol. This is even the case for inactive partial sequences of sCT (7). The phase transition of DMPG is also susceptible to perturbations by non-specific effects. At low ionic strength, for example, the transition is markedly broadened (8).

Despite the marked reduction and almost complete absence of lipid binding affinity, [Gly^8,Ala^{16}]-des-Leu^{19}-sCT still retains half of the biological activity of the native hormone as measured by *in vivo* plasma hypocalcemia in rats (9). Des-1-amino-[$Ala^{1,7}$,Gly^8]-des-Leu^{19}-sCT has an activity comparable to the native hormone despite its weak membrane affinity. Several factors affect the activity of multiple substituted analogues like [Gly^8, Ala^{16}]-des-Leu^{19}-sCT and des-1-amino-[$Ala^{1,7}$, Gly^8]-des-Leu^{19}-sCT. The principal conclusion from the present work is that these two analogues, with different modifications from the native hormone, both have substantial biological activity, despite an almost complete loss of lipid affinity. The peptides des-1-amino-[$Ala^{1,7}$]-des-Leu^{19}-sCT and [Gly^8]-des-Leu^{19}-sCT, which have an increased lipid affinity compared to des-1-amino-[$Ala^{1,7}$,Gly^8]-des-Leu^{19}-sCT and [Gly^8,Ala^{16}]-des-Leu^{19}-sCT, also have a higher biological activity at a level even greater than that of sCT. This enhanced activity above sCT may be due to increased conformational flexibility as previously discussed (10). Thus, a phospholipid-induced amphipathic helix can be an important structural feature increasing the lipid affinity and biological potency of membrane-active peptides. However, the present study clearly shows that this property is <u>not required for biological activity</u>.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

1. Epand, R. M., Jones, A. J. S., and Schreier, S. (1977) Biochim. Biophys. Acta 491, 296-304.
2. Kaiser, E. T. (1987) Biochem. Pharmacology 36, 783-788.

3. Epand, R. M., and Orlowski, R. C. (1987) Protides of the Biological Fluids 35, 481-484.
4. Green, F. R. III, Lynch, B., and Kaiser, E. T. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 8340-8344.
5. Epand, R. M., Gawish, A., Iqbal, M., Gupta, K. B., Chen, C. H., Segrest, J. P., and Anantharamaiah, G. M. (1987) J. Biol. Chem. 262, 9389-9396.
6. Orlowski, R. C., Epand, R. M., and Stafford, A. R. (1987) Eur. J. Biochem. 162, 399-402.
7. Epand, R. M., Stahl, G. L., and Orlowski, R. C. (1986) Int. J. Peptide Protein Res. 27, 501-507.
8. Epand, R. M., and Hui, S.-W. (1986) FEBS Lett. 209, 257-260.
9. Schwartz, K. E., Orlowski, R. C., and Marcus, R. (1981) Endocrinology 108, 831-835.
10. Epand, R. M., Epand, R. F., Orlowski, R. C., Seyler, J. K., and Colescott, R. L. (1986) Biochemistry 25, 1964-1968.