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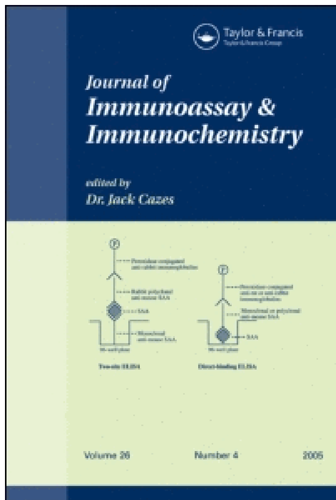

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A POSSIBLE BIOLOGICAL ROLE OF THE 'BIOLOGICALLY INACTIVE' REGION OF POLYPEPTIDE HORMONES

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ABSTRACT

In the cytochemical bioassay system, pentagastrin and the octapeptide of cholecystokinin were as potent as heptadecapeptide gastrin, on a molar basis. However, the degree of activation of parietal cell carbonic anhydrase, when tested in the presence of a low concentration of the heptadecapeptide, was only that expected for the heptadecapeptide alone. These results indicate that the supposedly biologically inactive region of the molecule may have a function in ensuring that the response of the target cells is specific to the natural hormone.

INTRODUCTION

The amino acid sequence of many polypeptide hormones can be divided into that sequence which evokes the biological response to the hormone, and the more species-specific 'tail' which apparently has no biological activity but is readily antigenic. For example, corticotrophin (ACTH) contains 39 amino acids but its full biological activity can be obtained with a synthetic α_{1-24} sequence common to all species of ACTH. Similarly, although human gastrin (G_{17}) is a heptadecapeptide, the first four C-terminal amino acids are the minimum required for the biological activity (1) so that synthetic 'pentagastrin' (PG; Peptavlon, I.C.I.) can be used therapeutically; equally, the C-terminal octapeptide (CCK-OP)

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of cholecystokinin, which has the same C-terminal four amino acids, will be expected to mimic G_{17}-gastrin.

Supraphysiological levels of gastrin (0.4 or 10 µg/100 g body weight) have been shown to stimulate carbonic anhydrase activity in rat gastric mucosa *in vivo* (2,3). A similar effect has been measured, by a quantitative cytochemical procedure (4), specifically in the parietal cells in strips of guinea-pig fundus maintained *in vitro* (5) and in suitably prepared sections of this tissue (6,7). The stimulation of activity was linearly related to the concentration of gastrin, from 2.3×10^{-12}M (low normal circulating level) to 2.3×10^{-15}M. This afforded a simple system for testing this aspect of the biological activity of the natural hormone and of its partial analogues.

One of the advantages of the cytochemical bioassay system is that each study, for example of the comparison of the relative potency of G_{17}-gastrin with that of CCK-OP and of PG, can be done on serial sections from one strip of fundus from one guinea-pig, so avoiding inter-animal variation.

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MATERIALS AND METHODS

The gastric fundus was removed from the guinea-pig (female: Hartley strain; about 400 g; killed by asphyxiation in nitrogen), cleared of debris and cut into strips that were then maintained for 5 h at 37°C in non-proliferative organ-culture (8). The strips were then exposed to a 'priming' concentration (2.3×10^{-16}M) of G_{17} for 5 min, to ensure full responsiveness, before being chilled to -70°C in *n*-hexane (6,7). Sections (18 µm thick) were cut in a cryostat (9) and these, in duplicate, were exposed for 75 s at 37°C to the various agents, this time having been found to give maximal stimulation, by these agents, of carbonic anhydrase activity in the parietal cells. Consequently the effects of these agents were compared in adjacent sections of the same region of the gastric fundus

in each guinea-pig used. Each comparative study was made on sections from one strip of fundus and was repeated four times using strips derived from two guinea-pigs.

The agents were dissolved in 0.1 M Hepes buffer, pH 7.0, containing 0.005% gum tragacanth. Carbonic anhydrase activity, visualized by the cytochemical reaction (4), was measured in individual parietal cells by scanning and integrating microdensitometry (4). The carbonic anhydrase activity was measured as the mean integrated extinction (8) of the reaction-product in the parietal cells. To test the reproducibility of the phenomena observed, each study was done five times. This entailed the use of tissue from four animals. Because of potential variation in the intrinsic responsiveness of each animal, the results have been expressed as the percentage stimulation of the carbonic anhydrase activity above that caused by $2.3x10^{-15}$M G_{17}-gastrin for each experiment in each animal.

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RESULTS

The stimulation in the parietal cells of carbonic anhydrase activity induced by increasing concentrations of Peptavlon (PG) was very similar to that found with G_{17}-gastrin. Thus in the assays done separately on two fundic strips from one animal and on three from another animal, the molar potency of Peptavlon relative to G_{17}-gastrin was 1.02±0.25 (SEM; n=5). However, in these five studies, when serial sections were exposed to PG at concentrations of 10^{-15} to 10^{-12} M but in the presence of $2.3x10^{-14}$M G_{17}, the amount of stimulation of carbonic anhydrase activity was equal to that caused by this concentration of the heptadecapeptide alone (Fig. 1). Thus at all these concentrations of PG, the percentage stimulation induced by these concentrations of PG acting in the presence of $2.3x10^{-14}$M G_{17}-gastrin was not statistically different from the stimulation induced by this concentration of G_{17}-gastrin acting alone.

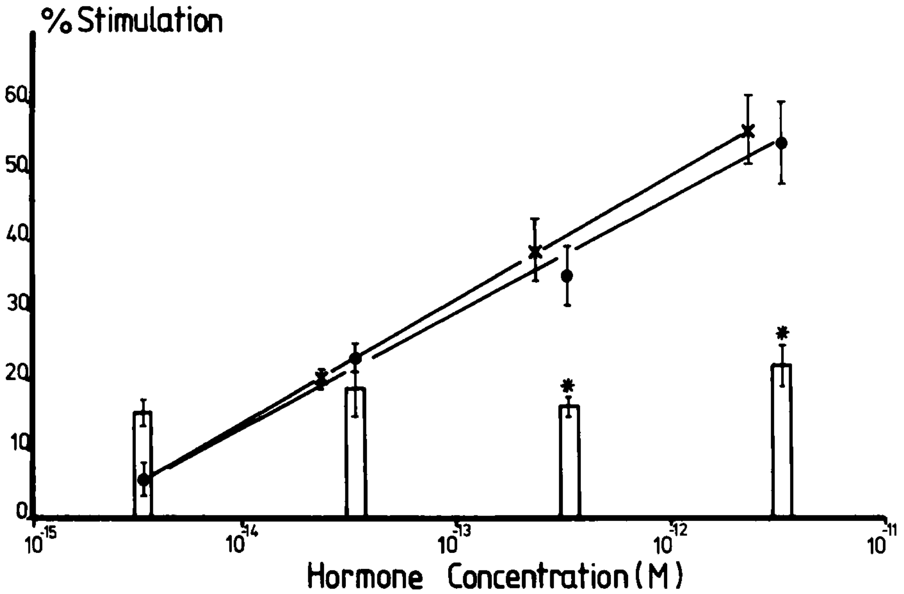

Fig. 1. The dose response graphs of G_{17} (x) and 'pentagastrin' (•) and the effect of the addition of 2.3×10^{-14}M G_{17} on the response to 'pentagastrin' (histograms). The results are expressed as the mean±SEM of the percentage stimulation of carbonic anhydrase activity over that elicited by 2.3×10^{-15}M G_{17} in 5 separate experiments on tissue from two different animals. The asterisks represent the concentration at which there was a significant difference between the 'pentagastrin' alone and 'pentagastrin' to which 2.3×10^{-14}M G_{17} had been added ($p<0.005$).

Similarly, the effect of four concentrations of G_{17}-gastrin and four concentrations of CCK-OP was measured in sections from one strip of fundus and repeated with sections of a second strip from the same animal, and with sections of three other strips from a second animal. In each of these comparative studies, the effect of adding the CCK-OP together with one concentration of the G_{17}-gastrin, was also measured.

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The response to the various concentrations of G_{17}-gastrin, in the sections from these two animals, was identical to that found previously (Table 1). The log dose-response graph for CCK-OP (over the range 10^{-15} to 10^{-12}M) was parallel to that for G_{17}-gastrin (Fig. 2); on a molar basis, the potency of the CCK-OP relative to the G_{17}-gastrin was 0.68±0.11 (SEM; n=5). However, when the stimulation of carbonic anhydrase activity was measured in serial sections that had been exposed to one concentration of G_{17}-gastrin (2.3×10^{-14}M) in the presence of one of a range of concentrations of CCK-OP (4.3×10^{-15} to 4.3×10^{-12}M), the stimulation was quantitatively identical with that found with 2.3×10^{-14}M G_{17}-gastrin alone (Fig. 2).

When one concentration of PG (6.3×10^{-14}M) was applied in the presence of one of a series of concentrations of CCK-OP (4.3×10^{-15} to 4.3×10^{-12}M) the amount of stimulation of the carbonic anhydrase activity rose with increasing concentrations of the CCK-OP (Fig. 3).

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Table 1. The response to various concentrations of G_{17}-gastrin in two separate series of experiments.

Concentration of G_{17} (M)	Percentage stimulation of carbonic anhydrase activity (Mean±SEM; n=5)	
	1st Series	2nd Series
2.3×10^{-14}	20.1 ± 1.6	22.7 ± 3.0
2.3×10^{-13}	38.4 ± 4.5	36.9 ± 4.5
2.3×10^{-12}	55.7 ± 5.0	57.7 ± 7.8

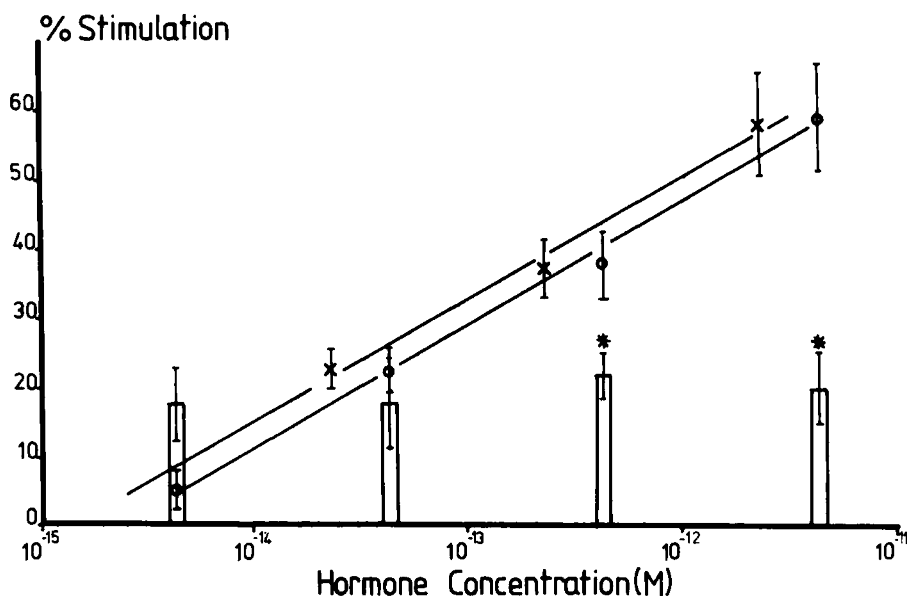

Fig. 2. The dose response graphs of G_{17} (x) and CCK-OP (o) and the effect of the addition of 2.3×10^{-14}M G_{17} on the response to CCK-OP (histograms). The results are expressed as the mean±SEM of the percentage stimulation of carbonic anhydrase activity over that elicited by 2.3×10^{-15}M G_{17} in 5 separate experiments on tissue from two different animals. The asterisks represent the concentrations at which there was a significant difference between the CCK-OP alone and CCK-OP to which 2.3×10^{-14}M G_{17} had been added ($p<0.005$).

DISCUSSION

Brown and Gallagher (10), in describing a specific gastrin receptor site in the rat stomach, showed that preincubation with 10^{3}M excess of 'pentagastrin' did not inhibit the binding of human gastrin I (G_{17}1) whereas a similar excess of either cholecystokinin or secretin inhibited the binding by at least 50%. Similarly Takeuchi et al. (11) found that 'pentagastrin' was one-tenth as potent as G_{17}-gastrin in causing 50%

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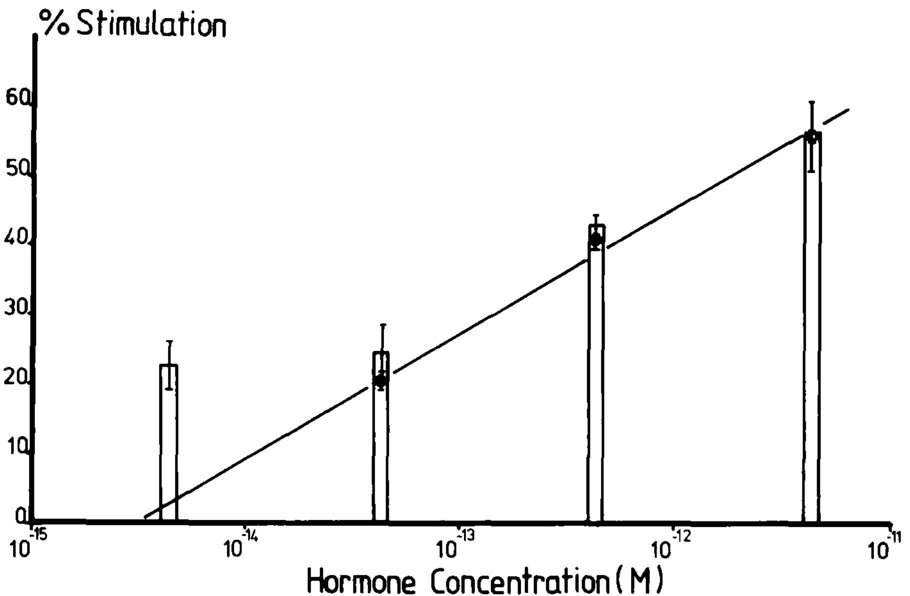

Fig. 3. The dose response graph to CCK-OP (o) and the effect of the addition of 6.3×10^{-14}M 'pentagastrin' (histograms). The results are expressed as the mean±SEM of the percentage stimulation of carbonic anhydrase activity over that elicited by 6.3×10^{-15}M CCK-OP in three separate experiments.

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inhibition of specific gastrin-binding to rat gastric membranes. Although the relative potency of 'pentagastrin' and of G_{17}-gastrin (or of cholecystokinin or of secretin) was not determined in these studies, the results are in accord with those presented in the present communication. In the present studies, the interaction with the receptor is inferred from the biological activity induced by these agents, acting either separately or in combination. The results suggest that the gastrin receptor may be capable of discriminating between the natural gastrin molecule and an homologous fragment, even when the latter is present in excess. This raises the possibility that the portion of the hormone-molecule that is normally considered

to be biologically inactive may have a function in ensuring that the response of the target-cell is specific to the natural hormone.

Acknowledgements

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