SMALL PEPTIDES WITH MELANOCYTE-STIMULATING ACTIVITY

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1. Introduction

Earlier investigations aiming at the elucidation of the relationship between the chemical structure and melanocyte-stimulating activity of α-melanotropin have led to the conclusion that the His–Phe–Arg–Trp amino acid sequence, occupying positions 6–9 in the hormone, possesses all qualitative properties necessary to elicit the specific biological activity [1].

Ac-Ser–Tyr–Ser–Met–Glu–His–Phe–

Arg–Trp–Gly–Lys–Pro–Val–NH_2

α-Melanotropin

Recently, Eberle and Schwyzer described the melanocyte-stimulating activity of the C-terminal tripeptide of the hormone, thus demonstrating the presence of two independent 'message sequences' in the polypeptide molecule [2].

In the course of a study on the biological properties of smaller synthetic melanotropin fragments, we found that not only the two peptides mentioned above, but a number of other partial sequences possess melanocyte-stimulating activity. Furthermore, naturally occurring peptides, such as enkephalins [3] and their fragments also exhibit a significant level of melanotropic potency. These unexpected findings make necessary a revision of the previous concept of specific hormone receptors and hormone active centers, at least as far as melanocyte-stimulating peptides are concerned.

2. Materials and methods

α-Melanotropin, enkephalins and their fragments were synthesized by the classical routes in this laboratory. Detailed account of these syntheses will appear elsewhere. All substances were checked for purity by chromatographic and electrophoretic methods, as well as by elemental and amino acid analysis.

Melanocyte-stimulating activity was determined by the in vitro frog skin assay developed by Shizume et al. [4], with slight modifications, using a Specol spectrophotometer with reflectometric accessories. Although *Rana pipiens* is commonly used as the test animal in the in vitro testing of MSH-activities, under our laboratory conditions *Rana esculenta* also proved to be suitable, giving, within the limits of error, the same activity values for a number of peptides as found in other laboratories. These data are also shown in table 1.

3. Results and discussion

The concept of the active centre or active sites in a polypeptide hormone is based mainly on the assumption that there must be a minimal sequence possessing the chemical and conformational features necessary to evoke a specific stimulus on the hypothetical hormone receptor. This sequence, a fragment of the whole hormone, should have a definite biological activity, which is qualitatively similar to that of the parent compound. This activity, sometimes only one-

Table 1

Peptide	MSH-units/mmol		
	Literature		Determined in this laboratory
α-Melanotropin	$4 \cdot 10^{10}$	[5]	$4 \cdot 10^{10}$
H-His–Phe–Arg–Trp–OH	$6 \cdot 10^{3}$	[1]	
H-Glu–His–Phe–Arg–Trp–Gly-OH	$2 \cdot 10^{5}$	[6]	$1 \cdot 10^{6}$
Ac-Lys–Pro–Val-NH_2	$8 \cdot 10^{4}$	[2]	$3 \cdot 10^{4}$
H-Ser–Tyr–Ser–Met-OMe			$2 \cdot 10^{4}$
H-Glu–His–Phe-OH			$1 \cdot 10^{4}$
H-Arg–Trp–Gly-OMe			$6 \cdot 10^{3}$
H-Lys–Pro–Val-NH_2	$3 \cdot 10^{4}$	[2]	
H-Glu–His–Phe–Lys–Pro–Val-NH_2			$1 \cdot 10^{5}$

millionth of that of the whole molecule, is enlarged by several orders of magnitude when this fragment is covalently combined with the rest of the molecule called binding sequences, reaching the highest potency in the complete molecule possessing the optimal structural features.

On the basis of this concept, two independent active sites have been postulated in α-melanotropin [2], as both the His–Phe–Arg–Trp tetrapeptide and the Lys–Pro–Val tripeptide are capable of producing melanine dispersion in the isolated frog skin. According to our present knowledge this would involve the existence of two adequate structure recognition sites on the melanotropin receptor.

This picture is, however, greatly complicated by our finding that not only the His–Phe–Arg–Trp and Lys–Pro–Val sequences, but also the N-terminal Ser–Tyr–Ser–Met tetrapeptide has a melanocyte-stimulating activity of its own. Furthermore, the Glu–His–Phe–Arg–Trp–Gly hexapeptide, believed to be biologically active only with the Phe–Arg bond being intact, could be separated into two active parts: the Glu–His–Phe and Arg–Trp–Gly tripeptides exhibit a melanocyte-stimulating potency of about the same magnitude as the active-site peptides mentioned previously (table 1).

That the recognition of a melanocyte-stimulating peptide by the melanocyte receptor, or in general, the melanotropic activity of low molecular weight peptides has to be explained on a different basis, is supported by some further observations. Thus enkephalins, naturally occurring peptides with potent opiate agonist activity, isolated recently from pig brain [3] showed a melanocyte-stimulating potency, which was comparable with that of the Glu–His–Phe–Arg–Trp–Gly hexapeptide derived from α-melanotropin. Furthermore, fragments of enkephalins, such as the Tyr–Gly–Gly

H-Tyr–Gly–Gly–Phe–Leu–OH

H-Tyr–Gly–Gly–Phe–Met-OH

Enkephalins

and Phe–Met sequences also proved to be active in the in vitro test, although their melanocyte-stimulating potency was smaller, and corresponded to the activity of the MSH tri- and tetrapeptides. These results are summarized in table 2.

In order to test whether these independently active small peptides are capable of producing higher biological activity in a cooperative manner when combined covalently, two derivatives were examined. The H-Glu–His–Phe–Lys–Pro–Val-NH_2 hexapeptide containing two low-acting melanotropin sequences had a melanocyte-stimulating activity ($1 \cdot 10^{5}$ U/mmol),

Table 2

Peptide	MSH-units/mmol
H-Tyr–Gly–Gly–Phe–Met-OMe	$5 \cdot 10^{5}$
H-Tyr–Gly–Gly–Phe–Leu-OH	$2.5 \cdot 10^{5}$
H-Tyr–Gly–Gly-OH	$2 \cdot 10^{4}$
H-Phe–Met-OMe	$1 \cdot 10^{4}$
H-Tyr–Gly–Gly–Lys–Pro–Val-NH_2	$5 \cdot 10^{5}$

which was higher than that of either of the components (table 1); the H-Tyr–Gly–Gly–Lys–Pro–Val-NH_2 hexapeptide, being the combination of an enkephalin and a melanotropin sequence, also proved to be more active than the constituting tripeptides (table 2).

It is fairly difficult to reach an unequivocal conclusion explaining the similar biological activity of a series of structurally unrelated peptides. Obviously, the qualification as an active site of the His–Phe–Arg–Trp or Lys–Pro–Val sequences may equally be attributed to other parts of the molecule. It is likely that a molecule possessing continuously rising biological potency can be built up from any of the active fragments, reaching the maximal activity in the intact α-melanotropin.

The fact that in α-melanotropin there are not only one or two, but at least four, and in other amino acid combinations possibly more 'active sites', can be explained in several ways. It is possible, for example, that the melanocyte-receptor does not possess any specific hormone recognizing region or, at least, this site is of a very limited specificity. In consequence, the receptor may interact with different peptides which are not structurally related to each other. As long as these peptides are small, they are bound to this nonspecific site loosely, and in order to get a reasonable amount of productive complexes and a measurable biological effect, an extremely high (up to 0.1 mmol) peptide concentration is necessary. With the growing complexity of the structure, a multiple-point attachment to the receptor through the (in themselves similarly nonspecific) peptide sequences can be attained, leading to a more stable complex with a higher specific activity. Finally, the optimal structure of the whole hormone, which corresponds the best to the adjacent binding sites on the receptor will result in the formation of the most productive hormone–receptor complex, accompanied by the highest biological potency. In other words, the intact hormone is a highly effective combination of low-specificity peptide parts, none of them playing a distinguished functional role.

It is also conceivable that while α-melanotropin initiates the series of events leading to the melanine dispersion by reacting with the receptor in a highly specific manner, the small hormone fragments and peptides not related to α-melanotropin interact with other, less specific sites. Then, of course, the question arises, which are the structural features necessary to distinguish between receptor sites of different specificity.

Finally, the possibility must not be left out of consideration that the effect of small peptides may manifest itself by some other mechanism than acting on the melanocyte receptor; melanine dispersion can also be produced by influencing other phases of this multistep process, such as inhibition of cyclic AMP degradation, alteration of ionic environment, etc. In this case, penetration of the small peptide into the inside of the cell has to be assumed, which might be the result of the high peptide concentration applied, approaching sometimes the ion concentration of the Ringer solution used in the in vitro experiments. Such a high peptide concentration, quite unusual in physiological conditions, may bring about melanocyte stimulation even by a nonspecific physical alteration of the membrane structure.

Further experiments are required to decide which of the possible mechanisms is responsible for the melanocyte-stimulating activity of these small peptides. It would also be worthwhile to investigate whether this phenomenon is restricted only to melanine dispersion, or can be found in the case of other peptide hormone actions.

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