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N-ACYLATED PENTAPEPTIDES ANTAGONISTS OF SUBSTANCE P ON GUINEA-PIG ILEUM

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SUMMARY: Pentapeptides X-D.Trp-Phe-D.Trp-Leu-Y-NH_2 (X = H, Boc, para-hydroxyphenylacetyl, Y = Met,Leu,Nle,Phe) were tested as antagonists against Substance P and against a specific agonist of the muscular receptor of neurokinins on the guinea-pig ileum . Weak antagonist or agonist activities could be observed with the free or the Boc-protected pentapeptides whilst the acylated compounds could be compared favorably with the best antagonists already described.

The three neurokinins identified in mammals : Substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) are widely distributed in the central and peripheral nervous system and are known to act on specific receptors to exert various biological functions (1,2). Both pharmacological and biochemical studies using neurokinin analogs, as well as their C-terminal biologically active fragments have permitted the identification of three distinct neurokinin receptors (3,4).

In view of the numerous roles attributed to these peptides in physiology and physiopathology (5,6), the synthesis of neurokinin antagonists provides a promising new type of pharmacological tool. Over the last five years, a fairly large number of antagonists analogs (undeca-, octa-, hepta- or hexapeptides) have been developed (7,8,9) and the common structural features, considered as minimal requirements for activity can be summarized as follows :

a/ the presence of at least two D-Trp residues generally at the positions 7 and 9;

b/ N-terminal residues replaced with D-isomers (D-Arg^1,D-Pro^2 in the undecapeptide, D-Pro^4 in the octapeptide) to protect the peptide from enzymatic degradation, or with L-isomers (Arg^1 in the hepta- and hexapeptides) to improve the solubility;

c/ C-terminal methionine changed for either an aliphatic residue ($NleNH_2$, $LeuNH_2$) or an aromatic residue ($PheNH_2$).

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Many reports have established that modifications starting from the C-terminal hexapeptide SP(6-11) sequence may lead to active agonists (10,11); these data seem to rule out the possibility that shorter fragments afford valuable agonists or antagonists. In fact, the C-terminal pentapeptide of neurokinins (Phe-X-Gly-Leu-MetNH$_2$, X = Phe, Val), although a weak agonist, is the minimal fragment able to promote a full spasmogenic activity on smooth muscle preparations. Extending the size of the fragments towards the N-terminus induces a rapid increase in potency. Larger fragments such as the heptapeptide SP(5-11) or the octapeptide SP(4-11) are more active than SP (12,13) and these results have been frequently explained by a privileged role of Gln^5 and Gln^6 in binding to the receptor (14). Interestingly, some N-acylated C-terminal pentapeptides SP(7-11) have revealed equal or even more activity than SP on smooth muscle (15) and it was thus tempting to evaluate N-acylated pentapeptides for their neurokinin antagonist activity.

In the present communication, we would like to discuss some data, which confirm the hypothesis that active antagonists can also be obtained by modification of a typical pentapeptide structure containing D-Trp residues in positions 7 and 9.

Isolated organs suspended *in vitro* are among the most useful pharmacological assays for characterizing new synthetic peptides,particularly antagonists, related to neurokinins. Activities were measured on the guinea-pig ileum in the presence of atropine against SP and against Septide : ($pGlu^6$,Pro^9) SP(6-11) (11).

MATERIALS AND METHODS

The SP pentapeptides antagonists (Table I) and Septide were prepared in our laboratory by the classic stepwise solution method and by fragment coupling (16). SP was purchased from Bachem, Switzerland.

Experiments were performed on guinea-pig ileum in the presence of atropine (5.2 µM). Segments of the ileum (2-3 cm length) were suspended in a 10 ml organ bath of Tyrode solution oxygenated by 95 % O_2, 5 % CO_2 at 37 °C (17). The preparation was kept under a resting load of 1 g and longitudinal contractions of the ileum were recorded isometrically after an equilibrium period of 60 min during which the bath medium was changed repeatedly. Before each test of a new analog, the peptide was tested alone to detect any spasmogenic activity. Then the antagonist was applied to the bath 5 min prior to the addition of the agonist (SP or Septide) ; maximum inhibition was observed in this interval.

Between the applications of agonists, a latency period of 15 min and careful washing cycles were performed to avoid tachyphylaxis and to overcome the inhibitory action of previously applied antagonists.

Affinities of antagonists were calculated in terms of pA_2 (-log of the concentration of antagonist that reduces the effect of a double dose of agonist to that of a single dose), according to Schild (18). Ratios of the response elicited by 8 nM agonist in the presence of antagonists versus the response produced by 4 nM agonist was plotted as a function of the logarithm of the antagonist concentration. Calculations were performed from a minimum of six experiments and values were given as means with 95 % confidence intervals.

RESULTS AND DISCUSSION

Results are summarized in Table 1 and indicate that :

- a/ all compounds are better antagonists against Septide than against SP;
- b/ N-protection with a Boc group does not result in great changes in activity;
- c/ the pentapeptide with D-Trp residues in positions 7 and 9 is, by itself, a weak antagonist (pA_2 near 5) and the N-acylation of the peptide leads to an important increase of the antagonist activities.

The primary objectives of this study were to examine to what extent a short C-terminal sequence in a neurokinin antagonist might be considered as the site of activity on the smooth muscle and to verify if the same acylations of the SP C-terminal pentapeptide would also result in some antagonist activities of the parent pentapeptide.

TABLE 1

Some Pentapeptides Antagonists

	COMPOUND	ACTIVITY ON GUINEA-PIG ILEUM (pA_2)[1]	
		against Septide[2]	against SP
1	DTrp-Phe-DTrp-Leu-Met-NH_2	5.34 ± 0.12	5.20 ± 0.32
2	DTrp-Phe-DTrp-Leu-Leu-NH_2	5.60 ± 0.05	5.05 ± 0.20
3	DTrp-Phe-DTrp-Leu-Nle-NH_2	5.94 ± 0.05	5.31 ± 0.18
4	DTrp-Phe-DTrp-Leu-Phe-NH_2	full agonist	
5	Boc[3]-DTrp-Phe-DTrp-Leu-Met-NH_2	5.26 ± 0.39	5.06 ± 0.32
6	Boc-DTrp-Phe-DTrp-Leu-Leu-NH_2	4.80 ± 0.30	4.75 ± 0.19
7	Boc-DTrp-Phe-DTrp-Leu-Nle-NH_2	5.64 ± 0.05	4.42 ± 0.25
8	Boc-DTrp-Phe-DTrp-Leu-Phe-NH_2	5.01 ± 0.17	4.54 ± 0.27
9	PHPA[4]-DTrp-Phe-DTrp-Leu-Met-NH_2	6.51 ± 0.21	5.69 ± 0.16
10	PHPA-DTrp-Phe-DTrp-Leu-Leu-NH_2	6.79 ± 0.21	5.73 ± 0.14
11	PHPA-DTrp-Phe-DTrp-Leu-Nle-NH_2	6.23 ± 0.12	5.72 ± 0.16
12	PHPA-DTrp-Phe-DTrp-Leu-Phe-NH_2	6.41 ± 0.07	5.72 ± 0.17

[1] pA_2 = -log of the concentration of antagonist that reduced the effect of a double concentration of agonist to that of a single dose.
[2] Septide = ($pGlu^6$,Pro^9)SP (6-11)
[3] Boc = tertiobutyloxycarbonyl -
[4] PHPA = 4-hydroxyphenylacetyl -

Of the more than one hundred neurokinin analog antagonists, the more efficient contain D-Trp residues in positions 7 and 9 or 7,9 and 10 (19) and methionine amide replaced by other hydrophobic aminoacid amides (8) . In this study, we found that pentapeptides X-D.Trp-Phe-D.Trp-Leu-Y-NH_2 (Y = Met, Leu,Nle,Phe) are weak antagonists whenever the N-terminus is free or blocked with a bulky and hydrophobic tertiobutyloxy radical (pA_2 near 5). We could also observe that this structure represents a minimum prerequisite for activity, since some pentapeptides act as very weak partial agonists (less than 5 % of the maximal activity at 10^{-6} M, for peptides 1,2 and 6) or as weak but full agonist (peptide 4).

The elongation of the pentapeptide antagonist minimal sequence towards the N-terminus with two aminoacid residues has been shown to improve the activity if this modification increases the solubility (9) and hexapeptide analogs containing N-methylated aminoacids and N-terminal Pro are more resistant to proteolysis and are better antagonists (pA_2 near 6.4)(20). In this study, we could confirm a previous observation that acylation of the C-terminal pentapeptide (15,16) by 4-hydroxyphenylacetic acid is a very reinforcing modification which exemplifies some important structural requirements controlling the affinity of the peptide for its binding sites. The gain in activity afforded by this acylating group is of the same order of magnitude as the two Gln residues in positions 5 and 6 of some synthetic antagonists (21).

The guinea-pig ileum, despite its lack of specificity (22), is the most commonly used pharmacological assay (7,23). In this preparation, atropine eliminates the cholinergic presynaptic component of the response to SP or to Septide, which has been reported as a selective agonist for the muscular receptor (11).

Our results, obtained against Septide with compounds 9-12, may thus be compared to those obtained on the same preparation against SP with an undecapeptide (D-Arg^1,D-$Trp^{7,9}$,Leu^{11}) SP = Spantide, pA_2 = 7.1) (24), with an octapeptide (D-Pro^4,D-$Trp^{7,9}$,Nle^{11}) SP(4-11), pA_2 = 7.1) (25) or with an heptapeptide (Arg^5,D-$Trp^{7,9}$,Nle^{11}) SP(5-11), pA_2 = 6.71) (26). Since activities of SP (ED_{50} = 1.75 10^{-9} M (7)) and of Septide (ED_{50} = 2.2 10^{-9} M (11)) are equivalent, the highest activities recorded against this latter agonist could be explained by a better selectivity of compounds 9-12 for a muscular receptor subtype (probably NK-1). Nevertheless, a selective degradation and/or different diffusion rates in the tissue may also account for this apparent selectivity.

It would be of great interest to compare these data to the affinities (K_I) in competition binding experiments performed with guinea-pig ileum (27). It appears unfortunately that naturally occuring peptides are

rather non selective for each receptor subtype. More selective radiolabelled ligands based on the structure of recently described selective agonists (28) should afford, when available, adequate correlations between pharmacological and biochemical data.

Further studies are in progress in our laboratory to define the new structural relationships implicated by these results.

REFERENCES

1. MAGGI C.A., GIULIANI S., SANTICIOLI P., REGOLI D. and MELI A.(1987) J.Auton.Pharmac. 7, 11-32.
2. PERNOW B. (1983) Pharmacological Review, 35, 85-141.
3. BUCK S.H., BURCHER E., SCHULTS C.W., LOVENBERG W. and O'DONOHUE T.L. (1986) Science 226, 987-999.
4. REGOLI D., DRAPEAU G., DION S. and D'ORLEANS-JUSTE P. (1987) Life Sciences 40, 109-117.
5. POST C.and FOLKERS K. (1985) Eur. J. Phamacol. 113,335-343.
6. LEANDER S., HAKANSON P., ROSELL S., FOLKERS K., SUNDLER F. and TORNQVIST K. (1981) Nature 294, 467-469.
7. REGOLI D., MIZRAHI J., D'ORLEANS-JUSTE P., DION S., DRAPEAU G. and ESCHER E. (1986) in Neural and Endocrine Peptides and Receptors (T.W.Moody Ed.) Plenum Press pp.501-511.
8. FOLKERS K., ROSELL S., JIE-CHENG X., BJORKROTH U., YI-AN L. and YIN-ZENG L. (1983) Acta Chim. Scand. 37B, 623-627.
9. HÖRIG J. and SCHULTHEISS H. (1984) Pharmacology 105, 65-72.
10. POULOS C., BROWN J.R. and JORDAN C.C. (1986) J.Med.Chem. 29, 1281-1284.
11. LAUFER R., GILON C., CHOREV M. and SELINGER Z.(1986) J.Med.Chem.29 1284-1288.
12. BURY R.W. and MASHFORD M.L. (1976) J. Med. Chem. 19, 854-856.
13. BERGMAN J., BIENERT M., NIEDRICH H., MEHLIS B. and P.OEHME P. (1974) Experientia 15(4), 401-403.
14. THEODOROPOULOS D., PINAS N., POULOS C., COUTURE R., MIZRAHI J., ESCHER E. and REGOLI D. (1982) Eur. J. Med. Chem. 17, 527-530.
15. BIENERT M., FORNER K., MEHLIS B. and NIEDRICH H. (1983) in Peptides 1982 (Blaha K.& Malon P. Ed.) Walter de Gruyter N.Y. p.517-520.
16. MICHELOT R., MAYER M., MAGNENEY S., PHAM VAN CHUONG P., SCHMITT P. and POTIER P. (1988) Eur. J. Med. Chem. 23, 243-247.
17. CALAS B., MICHELOT R., LECAER J.-P., CAVE A., PARELLO J. and POTIER P. (1987) Int. J. Peptide Protein Res. 29, 170-176.
18. SCHILD H.O. (1949) Brit. J. Pharmacol. 4, 277.
19. DUTTA A.S. (1987) Drugs Future 781-792.
20. BAIZMAN E.R., BENTLEY H., GORDON T.D., HANSEN P.E., KIEFER D., McKAY F.C., PERRONE M.H., MORGAN B.A., ROSI D., SINGH J., STEVENSON J. and WARD S.J. (1984) in Peptides (Ragnarsson U.E. Ed.) Almqvist and Wiksell international Stockholm p.359-364.
21. MATHISON R., ESCHER E., HUGGEL H., MIZRAHI J. and REGOLI D. (1985) Eur. J. Pharmacol.114, 147-155.
22. LEE C.M., CAMPBELL N.J., WILLIAMS B.J. and IVERSEN L.L. (1986) Eur. J. Pharmacol. 130, 209-217.
23. REGOLI D., D'ORLEANS-JUSTE P., ESCHER E. and MIZRAHI J. (1984) Eur. J. Pharmacol. 97, 161-170.
24. FOLKERS K., HAKANSON R., HÖRIG H., XU J.C. and LEANDER S. (1984) Br. J. Pharmacol. 83,449-456.
25. REGOLI D., ESCHER E. and MIZRAHI J. (1984) Pharmacology 28, 301-320
26. HÖRIG H. and SCHULTHEISS (1984) Eur. J. Pharmacol. 105, 65-72
27. BURCHER E.,BUCK S.H.,LOWENBERG W. and O'DONOHUE T.L. (1986) J. Pharmacol. Exp. Ther. 236, 819-831.
28. DRAPEAU G., D'ORLEANS-JUSTE P., DION S.,RHALEB N.-E., ROUISSI N.-E. and REGOLI D. (1987) Neuropeptides, 10, 43-54.