

more potent (2 to 50 times) than the (+)-isomer or the racemate. The greatest separation in the activity of the isomers was observed with MR 2266 in inhibiting the binding of [<sup>3</sup>H]MeTRH to brain TRH receptors. The stereoselectivity of the action of tifiuadom at TRH receptors also compares favourably at the opiate receptor in the absence of sodium (Kley et al 1983).

In summary, the present studies indicate that  $\kappa$ -opioid drugs inhibit the binding of [<sup>3</sup>H]MeTRH to brain receptors in a stereoselective manner with tifiuadom being the most potent agent. However, absolute stereospecificity is not exhibited by  $\kappa$ -opiate drugs in the above effect. These results help in further elucidating the biochemical mechanism for the in-vivo interaction between TRH and opiates.

These studies were supported by grant DA-02598 from the National Institute on Drug Abuse and by a Chicago Heart Association grant. The authors thank G. A. Matwyshyn for excellent technical assistance and Mr Pathrose Paulose for his help in the preparation of this manuscript.

### References

- Bhargava, H. N. (1980a) *Life Sci.* 29: 845–850  
 Bhargava, H. N. (1980b) *Psychopharmacology* 68: 185–189  
 Bhargava, H. N. (1981a) *Neuropharmacology* 20: 699–702  
 Bhargava, H. N. (1981b) *Life Sci.* 29: 1015–1020  
 Bhargava, H. N. (1981c) *Ibid.* 28: 1261–1267  
 Bhargava, H. N., Das, S. (1986) *Brain Res.* 368: 262–267  
 Bhargava, H. N., Matwyshyn, G. A. (1980) *Eur. J. Pharmacol.* 68: 147–154  
 Bhargava, H. N., Matwyshyn, G. A. (1985) *Psychopharmacology* 87: 141–146  
 Bhargava, H. N., Pillai, N. P. (1985) *Life Sci.* 36: 83–88  
 Bhargava, H. N., Yousif, D. J., Matwyshyn, G. A. (1983) *Gen. Pharmacol.* 14: 565–570  
 Bowers, C. Y., Friesen, H. G., Hwang, P., Guyda, H. J., Folkers, K. (1971) *Biochem. Biophys. Res. Comm.* 45: 1033–1041  
 Das, S., Bhargava, H. N. (1987) *Neuropharmacology*, 26: 1141–1146  
 Kley, H., Scheidemantel, U., Bering, B., Miller, W. E. (1983) *Eur. J. Pharmacol.* 87: 503–504  
 Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) *J. Biol. Chem.* 193: 265–275  
 Martin, B. R., Dewey, W. L., Chau, Pham, T., Prange, A. J. Jr. (1977) *Life Sci.* 20: 715–722  
 Nemeroff, C. B., Loosen, P. T., Bisette, G., Manberg, P. J., Wilson, I. C., Lipton, M. A., Prange, A. J. Jr. (1979) *Psychoneuroendocrinology* 3: 279–310  
 Pillai, N. P., Bhargava, H. N. (1984) *Peptides* 5: 1055–1059  
 Simasko, S. M., Horita, A. (1982) *Life Sci.* 30: 1793–1799  
 Tache, Y., Lis, M., Collu, R. (1977) *Ibid.* 21: 841–846

*J. Pharm. Pharmacol.* 1988, 40: 72–73  
 Communicated May 18, 1987

© 1988 J. Pharm. Pharmacol.

## Structure-activity relationship of hylambatin and its fragments as studied in the guinea-pig ileum

A. INOUE, T. FUKUYASU, Y. NAKATA, H. YAJIMA\*, M. NOMIZU†, Y. INAGAKI†, K. ASANO†, T. SEGAWA, *Department of Pharmacology, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, \*Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan, †Kirin Brewery Co., Ltd, Pharmaceutical Laboratory 2-2, Soujamachi 1-chome, Maebashi, Gunma 371, Japan*

**Abstract**—Hylambatin (Hyl), a dodecapeptide isolated from the skin of the African frog, *Hylambates maculatus*, belongs to the family of tachykinin or physalaemin-like peptides. Hylambatin and its 12 fragments were tested in the guinea-pig ileum preparation for contractile activities. All fragments except 3 had contractile activities. The C-terminal fragment as short as the octapeptide sequence was at least as active as the parent molecules. The heptapeptide fragment (Hyl<sub>6-12</sub>) and the hexapeptide fragment (Hyl<sub>7-12</sub>) were less active and the C-terminal pentapeptide fragment (Hyl<sub>8-12</sub>) and the N-terminal hexapeptide fragment (Hyl<sub>1-6</sub>) were much less active. The N-terminal pentapeptide fragment (Hyl<sub>1-5</sub>) and the N-terminal fragment from which the N-terminal Asp or Asp-Pro residues were removed (Hyl<sub>2-6</sub>, Hyl<sub>3-6</sub>), were inactive at doses used.

Yasuhara et al (1981) elucidated the structure of a new tachykinin peptide, named hylambatin, isolated from the skin of the South African rhacopharid frog (*Hylambates maculatus*). It is a physalaemin-like dodecapeptide and contains the C-terminal amino acid sequence common to all other tachy-

kinins replacing the Leu residue at position 2 from the C-terminal by a Met residue (Fig. 1). In parallel bioassay on smooth muscle preparations, blood pressure and salivary secretion, hylambatin and physalaemin were nearly indistinguishable from each other (Falconi Erspamer et al 1984). Hylambatin injected intravenously significantly increased both plasma glucose and plasma insulin in rats (Güllner et al 1984). In guinea-pig ileum, hylambatin was 0.42 times less active than kassinin (Okamoto et al 1984). In the present work, to elucidate the structure-activity relationship, the contractile activities of hylambatin and its fragments were examined in the guinea-pig ileum.

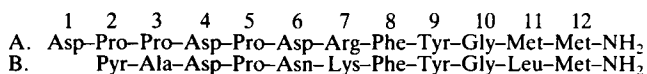


Fig. 1. Structures of (A) hylambatin and (B) physalaemin.

### Materials and methods

The guinea-pig ileum was mounted in a 20 mL organ bath containing Tyrode solution thermostated at 32 °C and bubbled

Correspondence to: T. Segawa, Dept of Pharmacology, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Kasumi 1-2-3, Minami-ku, Hiroshima 734, Japan.

Table 1. Structures and contractile potency in guinea-pig ileum of hylambatin fragments. EC50 values indicate the concentration of the peptides to give half maximal contraction. Ratio means the relative potency of the peptides with respect to that of parent hylambatin (taken as 1).

Peptide	1	2	3	4	5	6	7	8	9	10	11	12	EC50 (M)	Ratio
Hylambatin	Asp-Pro-Pro-Asp-Pro-Asp-Arg-Phe-Tyr-Gly-Met-Met-NH <sub>2</sub>												$2 \times 10^{-9}$	1
Hyl <sub>1-6</sub>	Asp-Pro-Pro-Asp-Pro-Asp-OH												$> 10^{-5}$	—
Hyl <sub>1-5</sub>	Asp-Pro-Pro-Asp-Pro-OH												inactive	—
Hyl <sub>2-6</sub>	Pro-Pro-Asp-Pro-Asp-OH												inactive	—
Hyl <sub>3-6</sub>	Pro-Asp-Pro-Asp-OH												inactive	—
Hyl <sub>2-12</sub>	Pro-Pro-Asp-Pro-Asp-Arg-Phe-Tyr-Gly-Met-Met-NH <sub>2</sub>												$2 \times 10^{-9}$	1
Hyl <sub>3-12</sub>	Pro-Asp-Pro-Asp-Arg-Phe-Tyr-Gly-Met-Met-NH <sub>2</sub>												$2 \times 10^{-9}$	1
Hyl <sub>4-12</sub>	Asp-Pro-Asp-Arg-Phe-Tyr-Gly-Met-Met-NH <sub>2</sub>												$2 \times 10^{-9}$	1
Hyl <sub>5-12</sub>	Pro-Asp-Arg-Phe-Tyr-Gly-Met-Met-NH <sub>2</sub>												$2 \times 10^{-9}$	1
Hyl <sub>6-12</sub>	Asp-Arg-Phe-Tyr-Gly-Met-Met-NH <sub>2</sub>												$1 \times 10^{-8}$	1/5
Hyl <sub>7-12</sub>	Arg-Phe-Tyr-Gly-Met-Met-NH <sub>2</sub>												$1 \times 10^{-8}$	1/5
Hyl <sub>8-12</sub>	Phe-Tyr-Gly-Met-Met-NH <sub>2</sub>												$1 \times 10^{-5}$	1/5000
Hyl <sub>9-12</sub>	Tyr-Gly-Met-Met-NH <sub>2</sub>												$> 10^{-5}$	—

with air. Isotonic contractions in response to peptides were measured with the aid of an isotonic transducer (KN259 Natsume Seisakusho Co., Ltd) and recorded in a recticorder (KN260 Natsume Seisakusho Co., Ltd). Chemical synthesis of hylambatin and its fragments will be described in detail elsewhere. All peptides were dissolved in H<sub>2</sub>O and diluted serially.

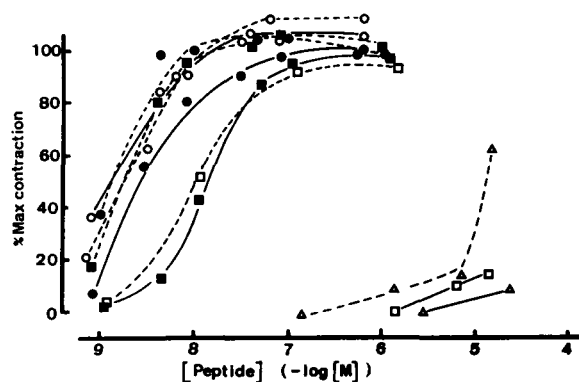


FIG. 2. Representative concentration-response curve for hylambatin fragments-induced contractions of guinea-pig ileum. Symbols are as follows: hylambatin —●—, Hyl<sub>1-6</sub> —□—, Hyl<sub>2-12</sub> —○—, Hyl<sub>3-12</sub> —○—, Hyl<sub>4-12</sub> —■—, Hyl<sub>5-12</sub> —●—, Hyl<sub>6-12</sub> —■—, Hyl<sub>7-12</sub> —□—, Hyl<sub>8-12</sub> —△—, Hyl<sub>9-12</sub> —△—.

### Results and discussion

The structures of hylambatin fragments used and the potency pattern of them are summarized in Table 1. The minimum sequence requirement for biological activity is represented by the C-terminal pentapeptide (including Phe). Shortening the hylambatin chain down to the C-terminal hexapeptide maintains full activity or at least 20% of the activity of the dodecapeptide. N-terminal fragments are all inactive. Plotting of the relative contraction (% maximum contraction) against log molar concentration of the peptides resulted in S-shaped curves, which is shown in Fig. 2.

Thus, the structure-activity relationship shows that C-terminal residue of hylambatin is essential for the contractile activity on guinea-pig ileum preparation.

### References

- Falconi Erspamer, G., Mazzanti, G., Yasuhara, T., Nakajima, T. (1984) *J. Pharm. Pharmacol.* 36: 284-285
- Güllner, H.-C., Harris, V., Yajima, H., Unger, R. H. (1984) *Arch. Int. Pharmacodyn.* 272: 304-309
- Okamoto, K., Yasumura, K., Fujitani, K., Katakura, S., Akaji, K., Yajima, M., Nakata, Y., Inoue, A., Segawa, T. (1984) *Chem. Pharm. Bull.* 32: 430-437
- Yasuhara, T., Nakajima, T., Falconi Erspamer, G., Erspamer, V. (1981) *Biomedical Res.* 2: 613-617