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Stabilization of thymopentin and preservation of its pharmacological properties by 2-hydroxypropyl-β-cyclodextrin

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Abstract—Thymopentin prepared in 5, 15, and 20% 2-hydroxypropyl-β-cyclodextrin (HPCD) was able to inhibit guinea-pig ileum contraction stimulated by anatoxin-a (3×10^{-6} M) after fourteen months of storage at room temperature. Thus, in contrast to the instability of thymopentin prepared without HPCD, the pharmacological activity was retained and could be stored in a ready-to-use solution for extended periods without refrigeration.

Thymopentin (TP-5; Arg-Lys-Asp-Val-Tyr), a synthetic peptide corresponding to position 32–36 of thymopoietin, is effective in blocking the stimulation of smooth-muscle contraction by the neurotoxin (+)-anatoxin-a (Chiang et al 1991). Anatoxin-a is a bicyclic amine exotoxin produced by the blue green algae, *Anabaena flos-aquae* (Carmichael et al 1975), and has been found to cause death to livestock and waterfowl via a depolarizing blockade of neuromuscular transmission, and subsequent respiratory paralysis (Carmichael et al 1975; Devlin et al 1977). The toxic action has been ascribed to its potent nicotinic cholinergic agonist activities in skeletal muscle (Spivak et al 1980), *Torpedo* electric organ (Aronstam & Witkop 1981) and mammalian central nervous system (Zhang et al 1987). Anatoxin-a can also induce cardiovascular effects by the activation of nicotinic receptors in the adrenal medulla and sympathetic ganglia (Siren & Feuerstein 1990). The antagonist effect of thymopentin has been attributed to its ability to block nicotinic receptors in a noncompetitive manner (Ochoa et al 1990; Chiang et al 1991).

One drawback in the use of peptides such as thymopentin in the treatment of diseases and poisoning, is that peptides are usually hydrolytically unstable in aqueous solution, and ordinarily cannot be stored for extended periods in their ready-to-use form (Septov et al 1991). At present, there is no known compatible solvent in which thymopentin is hydrolytically stable. This is a significant problem because the instability of medically important peptides will hinder their acceptance for prophylactic or therapeutic applications. Cyclodextrins can be utilized to form stable inclusion complexes with hydrolytically unstable peptides (Brewster et al 1991; Strattan 1992a,b). Cyclodextrins are homologous cyclic molecules containing six or more α-D-glucopyranose units linked together at the 1,4 pos-

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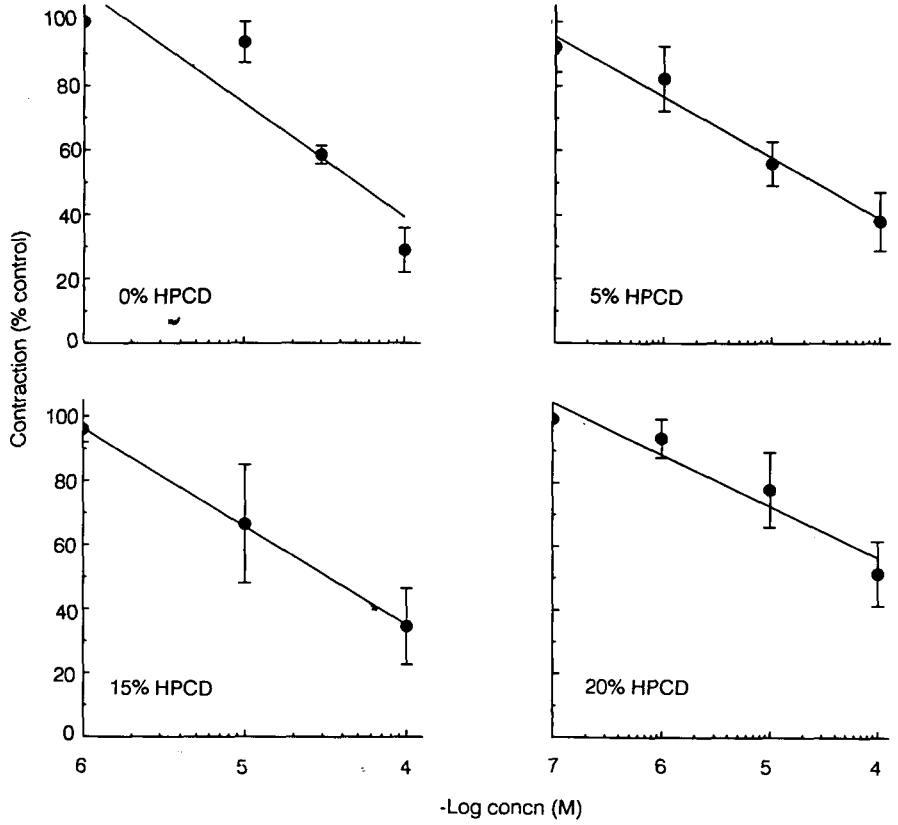

FIG. 1. Inhibition by thymopentin of guinea-pig ileum contraction stimulated by anatoxin-a (3×10^{-6} M) in 5, 15 and 20% HPCD after fourteen months of storage at room temperature (25°C). Each point represents mean ± s.e. of four separate experiments.

Table 1. IC50 values of the inhibition by thymopentin of guinea-pig ileum contraction stimulated by anatoxin-a (3×10^{-6} M) in 5, 15 and 20% HPCD after fourteen months of storage at room temperature (25°C). Each point represents mean ± s.e. of four separate experiments.

HPCD (%)	IC50 (10^{-5} M)
0 (freshly made)	3·9 ± 1·9
5	4·1 ± 3·1
15	4·9 ± 4·4
20	3·3 ± 3·0

itions as in amylose. The six-unit cyclodextrin is known as α-cyclodextrin, the seven-unit cyclodextrin is known as β-cyclodextrin, and the eight-unit cyclodextrin is known as γ-cyclodextrin. The use of 2-hydropropyl-β-cyclodextrin (HPCD) as a solubilizing and stabilizing excipient for protein drugs has been reported (Brewster et al 1991). This communication therefore examines the use of HPCD to stabilize thymopentin in solution.

Materials and methods

HPCD was purchased from Pharmatec, Alachua, FL. Thymopentin (10^{-2} M), synthesized as described previously (Chiang et al 1991), was made up in various percentages of HPCD dissolved in distilled water. Mixtures were stirred for about one hour. The solutions were then maintained at room temperature (25°C). Control solutions of thymopentin dissolved in distilled water without HPCD were also prepared in the same manner. The two sets of solutions were stored at ambient room temperatures for fourteen months. Aliquots (100 μL) were removed monthly for stability testing. The stability study was performed by assaying the ability of the thymopentin solutions to counteract the stimulation of contraction of guinea-pig ileum by anatoxin-a. Guinea-pig ileum contraction stimulated by anatoxin-a was performed as reported (Gordon et al 1989; Chiang et al 1991). The final concentration of thymopentin used was obtained by dilution with Krebs–Ringer buffer.

Results and discussion

Fig. 1 shows that thymopentin in 5, 15 and 20% HPCD, when tested on guinea-pig ileum, was pharmacologically active as an antagonist of anatoxin-a after storage for fourteen months at room temperature (25°C). Thymopentin in each HPCD preparation yielded similar ED50 values in antagonizing the stimulatory action of anatoxin-a on guinea-pig ileum (Table 1). In contrast, thymopentin prepared without HPCD showed no antagonist activity after one week of storage at room temperature (not shown). Our data established that thymopentin was stabilized with HPCD and could be stored in ready-to-use solution at room temperature for extended time periods without degradation. The present investigation also demonstrated the feasibility of HPCD for stabilization of peptide drugs in a non-toxic carrier, following its delivery to pharmacological targets.

References

Aronstam, R. S., Witkop, B. (1981) Anatoxin interaction with cholinergic synaptic molecules. Proc. Natl. Acad. Sci. USA 78: 4639–4643

Brewster, M. E., Hora, M. S., Simpkins, J. W., Bodor, N. (1991) Use of 2-hydroxypropyl-β-cyclodextrin as solubilizing and stabilizing excipient for protein drugs. Pharm. Res. 8: 792–795

Carmichael, W. W., Biggs, D. F., Gorham, P. R. (1975) Toxicology and pharmacological action of *Anabaena flos-aquae* toxin. Science 187: 542–544

Chiang, P. K., Butler, D. L., Brown, N. D. (1991) Nicotinic action of anatoxin-a on guinea pig ileum antagonized by thymopentin. Life Sci. 49: PL13–19

Devlin, J. P., Edwards, O. E., Gorham, P. R., Hunter, N. R., Pike, R. K., Stavric, B. (1977) Anatoxin-a, a toxic alkaloid from *Anabaena flosaquae* NRC-44h. Can. J. Chem. 55: 1367–1371

Gordon, R. K., Breuer, E., Padilla, F. N., Smejkal, R. M., Chiang, P. K. (1989) Distance geometry of α-substituted 2,2-diphenylpropionate antimuscarinics. Mol. Pharmacol. 36: 766–772

Ochoa, E. L. M., Li, L., Plummer, A., McNamee, M. G. (1990) Direct effects of thymopentin (Arg-Lys-Asp-Val-Tyr) on cholinergic agonist-induced slow inactivation of nicotinic acetylcholine receptor function. Mol. Pharmacol. 38: 863–871

Septov, N. F., Krymsky, M. A., Ovchinnikov, M. V., Bespalova, Z. D., Isakova, O. L., Soucek, M., Lebl, M. (1991) Rearrangement, racemization and decomposition of peptides in aqueous solution. Peptide Res. 4: 308–312

Siren, A.-L., Feuerstein, G. (1990) Cardiovascular effects of anatoxin-a in the conscious rat. Toxicol. Appl. Pharmacol. 102: 91–100

Spivak, C. E., Witkop, B., Alburquerque, E. X. (1980) Anatoxin-a: a novel agonist at the nicotinic receptor. Mol. Pharmacol. 18: 384–394

Strattan, C. E. (1992a) 2-Hydroxypropyl-β-cyclodextrin: part I, patents and regulatory issues. Pharmaceut. Technol. Jan. 68–74

Strattan, C. E. (1992b) 2-Hydroxypropyl-β-cyclodextrin: part II, safety and manufacturing issues. Pharmaceut. Technol. Feb. 52–58

Zhang, X., Stjernlof, P., Adem, A., Nordberg, A. (1987) Anatoxin-a: a potent ligand for nicotinic cholinergic receptors in rat brain. Eur. J. Pharmacol. 135: 457–458