Modification of effects of biologically active peptides, caused by enzyme treatment, on the excitability of identifiable giant neurones of an African giant snail (Achatina fulica Férussac)

H. Takeuchi, M. Matsumoto and A. Mori[1]

Institute for Neurobiology, Okayama University Medical School, Okayama (Japan), 13 July 1976

Summary. Physalaemin, which excites an identifiable molluscan giant neurone (the TAN, tonically autoactive neurone), lost the effect after the trypsin treatment. Unexpectedly, this peptide shows an inhibitory effect on the same neurone after chymotrypsin treatment. Deamino-dicarba-(d-d-)oxytocin and d-d-Arg-vasotocin, which excite another identifiable neurone (the PON, periodically oscillating neurone) continue to show the effect after chymotrypsin treatment (6 h). But d-d-Arg-vasotocin losts the effect on the PON after trypsin treatment.

It was reported previously[2,3] that the excitability of 2 identifiable giant neurones[4,5] of an African giant snail (Achatina fulica Férussac) was affected by the application of some biologically active peptides as follows: physalaemin[6–8] showed a biphasic effect on the TAN (tonically autoactive neurone) (the excitatory effect was much more remarkable in this case), and deamino-dicarba-(d-d-)oxytocin[9] and d-d-Arg-vasotocin[10] showed an excitatory effect on the PON (periodically oscillating neurone). In the present study, we attempted to examine the modification of effects of these peptides, caused by treatment with chymotrypsin or trypsin, on the excitability of the 2 identifiable giant neurones.

To treat peptides with enzymes, the following substances dissolved in the snail's physiological solution (pH 7.5) were prepared: peptides to be treated (products of Protein Research Foundation, Osaka, dissolved at 2.5×10^{-4} kg/l), α-chymotrypsin (Worthington Biochemical Corporation, 55 U/mg, dis. at 5×10^{-5} kg/l) and TPCK (L-1-tosylamide-2-phenylethyl-chloromethylketone)treated trypsin (Worthington Biochemical Corporation, 266 U/mg, dis. at 5×10^{-5} kg/l). 1.0 ml of a peptide solution was incubated with 0.1 ml of an enzyme solution at 37 °C for 6 h. After incubation, a pancreatic trypsin-chymotrypsin inhibitor solution (Trasylol, Bayer AG, dis. at 2×10^{-5} kg/l) of about 0.17 ml was added. Consequently, the solution to be examined contained the original peptide at 2×10^{-4} kg/l. These solutions were administered by the bath application to the dissected ganglia. The intracellular biopotentials of the 2 giant neurones were recorded with a glass micropipette, and the number of their spike discharges per min counted by a spike counter.

1 The authors thank Dr Sadaaki Iwanaga of Osaka University and Dr Atsuo Inoue of Daiichi Pharmaceutical Co. for their helpful advice, and Miss Hiroko Tamura for her technical assistance.

2 H. Takeuchi, I. Yokoi and A. Mori, Experientia *32*, 606 (1976).
3 H. Takeuchi, A. Sakai and A. Mori, Experientia *32*, 1554 (1976).
4 H. Takeuchi, I. Yokoi, A. Mori and M. Kohsaka, Gen. Pharmac. *6*, 77 (1975).
5 H. Takeuchi, I. Yokoi, A. Mori and S. Ohmori, Brain Res. *103*, 261 (1976).
6 V. Erspamer, G. Bertaccini and J. M. Cei, Experientia *18*, 562 (1962).
7 V. Erspamer, A. Anastasi, G. Bertaccini and J. M. Cei, Experientia *20*, 489 (1964).
8 A. Anastasi, V. Erspamer and J. M. Cei, Arch. Biochem. Biophys. *108*, 341 (1964).
9 T. Yamanaka, S. Hase, S. Sakakibara, I. L. Schwartz, B. M. Dubois and R. Walter, Mol. Pharmac. *6*, 474 (1970).
10 S. Hase, S. Sakakibara, M. Wahrenburg, M. Kirchberger, I. L. Schwartz and R. Walter, J. Am. chem. Soc. *94*, 3590 (1972).

Modification of effects of biologically active peptides, when treated by chymotrypsin (CT) or trypsin (T) for 6 h, on the excitability of 2 identifiable giant neurones (the TAN, tonically autoactive neurone; and the PON, periodically oscillating neurone) of Achatina fulica Férussac (bath application at 2×10^{-4} kg/l). E, excitatory effect, I, inhibitory effect, (–), no effect. ↓CT, main sites cleaved by CT. ↓T, main sites cleaved by T. (Pyr: L-pyroglutamic acid; Asu: α-amino suberic acid)

Substance	Effects on TAN	Effects on PON	Amino acid sequence and main sites cleaved by enzymes
1 Physalaemin (non-treated)	E	(–)	T CT CT ↓ ↓ ↓ Pyr-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2
2 CT-treated (6 h)	I	(–)	
3 T-treated (6 h)	(–)	(–)	
4 Deamino-dicarba-oxytocin (non-treated)	(–)	E	CT ↓ ⌐→Tyr-Ile-Gln-Asn-Asu-Pro-Leu-Gly-NH_2 └──────┘
5 CT-treated (6 h)	(–)	E	
6 Deamino-dicarba-Arg-vasotocin (non-treated)	(–)	E	CT T ↓ ↓ ⌐→Tyr-Ile-Gln-Asn-Asu-Pro-Arg-Gly-NH_2 └──────┘
7 CT-treated (6 h)	(–)	E	
8 T-treated (6 h)	(–)	(–)	
9 Deamino-dicarba-Arg-vasopressin (non-treated)	(–)	(–)	CT CT T ↓ ↓ ↓ ⌐→Tyr-Phe-Gln-Asn-Asu-Pro-Arg-Gly-NH_2 └──────┘
10 CT-treated	(–)	(–)	
11 T-treated	(–)	(–)	

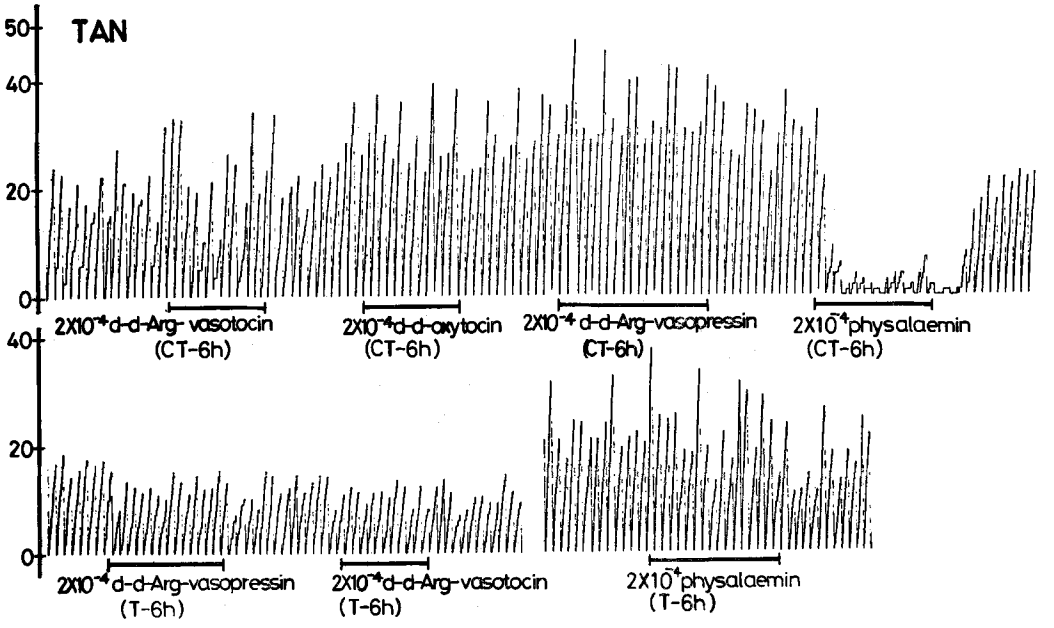

Fig. 1. Effects of biologically active peptides treated by enzymes, on TAN (tonically autoactive neurone) excitability (bath application). The upper trace was recorded from 1 TAN, and the lower trace from 2 TANs. Ordinate, the number of spike discharges per min. Abscissa, time course, each histogram is 1 min. We applied 2×10^{-4} kg/l deamino-dicarba-(d-d-)Arg-vasotocin (chymotrypsin [CT]-treated, 6 h), d-d-oxytocin (CT-treated, 6 h), d-d-Arg-vasopressin (CT-treated, 6 h), physalaemin (CT-treated, 6 h), d-d-Arg-vasopressin (trypsin [T]-treated, 6 h), d-d-Arg-vasotocin (T-treated, 6 h) and physalaemin (T-treated, 6 h). Note that physalaemin, when treated by T, lost its excitatory effect on the TAN; and that physalaemin treated by CT showed an inhibitory effect on the same neurone, opposite to that of untreated physalaemin.

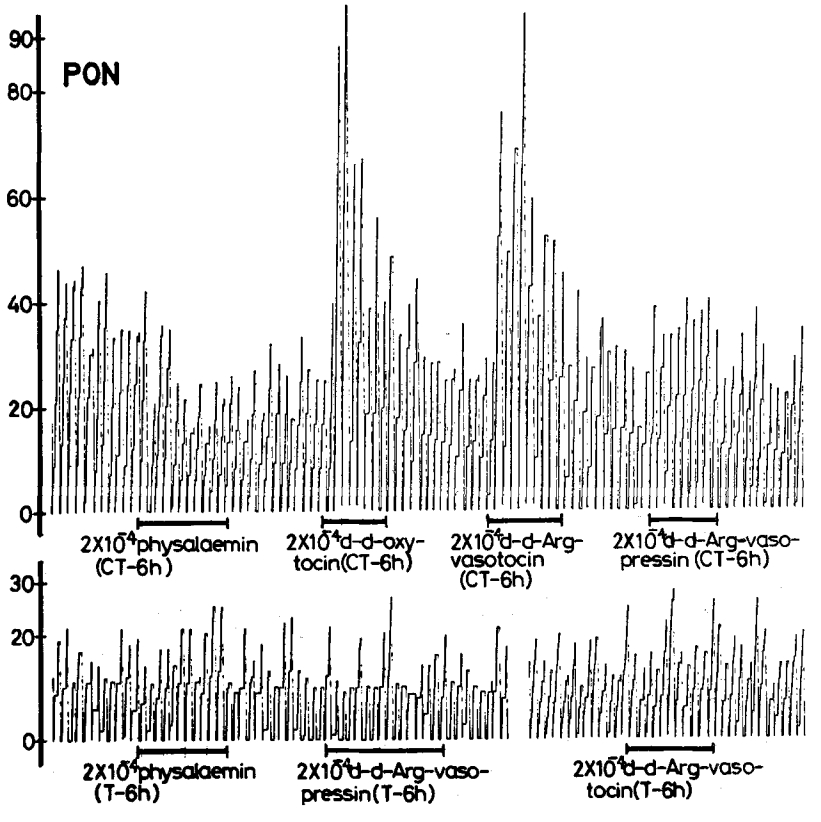

Fig. 2. Effects of biologically active peptides treated by enzymes on PON (periodically oscillating neurone) excitability (bath application). The upper trace was recorded from 1 TAN, and the lower trace from 2 TANs. Ordinate, the number of spike discharges per min. Abscissa, time course, each histogram is 1 min. We applied 2×10^{-4} kg/l physalaemin (chymotrypsin [CT]-treated, 6 h), deamino-dicarba(d-d-)oxytocin (CT-treated, 6 h), d-d-Arg-vasotocin (CT-treated, 6 h), d-d-Arg-vasopressin (CT-treated, 6 h), physalaemin (trypsin [T]-treated, 6 h), d-d-Arg-vasopressin (T-treated, 6 h) and d-d-Arg-vasotocin (T-treated, 6 h). Note that d-d-oxytocin and d-d-Arg-vasotocin, after CT-treatment for 6 h continued to show their excitatory effect on the PON; and that the excitatory effect of d-d-Arg-vasotocin disappeared after T-treatment.

Effects of four peptides (physalaemin, d-d-oxytocin, d-d-Arg-vasotocin and d-d-Arg-vasopressin), when treated by chymotrypsin or trypsin, on the excitability of 2 giant neurones are summarized in the table, in comparison with those of these peptides untreated. In these cases, we examined solutions of these peptides in concentrations of 2×10^{-4} kg/l.

Figure 1 demonstrates effects of these enzyme-treated peptides on TAN excitability. Physalaemin lost its excitatory effect on this neurone after trypsin treatment. Physalaemin, when treated by chymotrypsin, unexpectedly showed an inhibitory effect on the same neurone, opposite to that of untreated physalaemin. 3 peptides analogous to neurohypophyseal hormones (d-d-oxytocin, d-d-Arg-vasotocin and d-d-Arg-vasopressin) had no effect on the TAN, whether treated or not.

Figure 2 shows effects of these treated peptides on the PON. D-d-oxytocin and d-d-Arg-vasotocin continued to show their excitatory effect on the PON after chymotrypsin treatment for 6 h. However, d-d-Arg-vasotocin lost its effect after trypsin treatment. Physalaemin and d-d-Arg-vasopressin, whether treated or not, had no effect on the PON.

In spite of its remarkable excitatory effect of physalaemin on the TAN[2], this substance, when treated by trypsin, no longer showed its effect. As shown schematically in the table, trypsin must cleave the peptide bond of 'Phe-Lys' of physalaemin. Konishi and Otsuka[11] reported that several hypotensive peptides including physalaemin commonly affected the ventral root potential of the frog spinal cord, and assumed that a common C-terminal sequence (-Phe-X-Gly-Leu-Met-NH_2) of these peptides caused the depolarization of spinal motoneurones. We could not confirm their hypothesis with our experimental material, since a fragment of physalaemin, 'Phe-Tyr-Gly-Leu-Met-NH_2', which is considered to be produced by trypsin treatment, showed no effect on the TAN. We can say that a certain amino acid sequence of physalaemin, longer than the above-mentioned fragment, is necessary to produce the effect of untreated physalaemin on this neurone. After treatment with chymotrypsin, not only physalaemin's excitatory effect on the TAN disappeared, but also an inhibitory effect on the same neurone was apparent. We are convinced that some new peptide showing the inhibitory effect on the TAN was produced by the chymotrypsin treatment, although we cannot conclude whether inhibitory active sites of this new peptide are identical with those of untreated physalaemin in producing the slight inhibitory effect on the same neurone.

Previously we reported[3] that d-d-oxytocin and d-d-Arg-vasotocin, but not d-d-Arg-vasopressin, showed an excitatory effect on the PON. Trypsin treatment of d-d-Arg-vasotocin made its effect disappear. Since trypsin must cleave the peptide bond of 'Arg-Gly' of this substance, glycylamine (Gly-NH_2) in the C-terminal is indispensable in producing the effect. On the other hand, 'Ile' in the second position of the amino acid sequence of this substance is also indispensable to produce the effect, since d-d-Arg-vasopressin, having 'Phe' instead of 'Ile' of d-d-Arg-vasotocin, showed no effect on the same neurone. Therefore, it is assumed that almost complete amino acid sequences of d-d-oxytocin and d-d-Arg-vasotocin are necessary to produce the effect on the PON. After the chymotrypsin treatment for 6 h in the condition described above, the 2 peptides analogous to neurohypophyseal hormones continued to show their effect. The amino acid sequences of d-d-oxytocin, d-d-Arg-vasotocin and physalaemin necessary to produce effects on our experimental materials have to be ascertained in a further study.

11 S. Konishi and M. Otsuka, Brain Res. *65*, 397 (1974).

Development of noradrenaline uptake in the human foetal heart

S. Saarikoski[1]

Department II of Obstetrics and Gynaecology, University Central Hospital, SF-00290 Helsinki 29 (Finland),
20 July 1976

Summary. The development of NA-uptake mechanisms in the human foetal heart start at the same time as the adrenergic terminals were visible. The highest ^{3}H-NA values in the human foetal heart were only 25–30% of those found in the mouse heart.

The adrenergic nervous system develops later in the human foetal heart than in many other peripheral tissues according to mainly morphological examinations[2–7]. Present knowledge of the functional development of the adrenergic nervous system in the human foetal heart is more limited. The adrenergic receptors are believed to respond at 9 weeks of gestation to adrenaline[8], but not before 13 weeks of gestation to field stimulation[9], and the metabolic inactivation mechanisms are believed to be of more significance than the uptake mechanisms in the second trimester of pregnancy[10].

In the present work the functional development of the adrenergic nervous system was examined by estimating the ^{3}H-noradrenaline (^{3}H-NA) uptake in the isolated atria and ventricles of human foetal heart as compared with the mouse atria and ventricles, and with the development of the adrenergic nerve fibres observed histochemically.

Material and methods. Foetal hearts were obtained from legal terminations of pregnancy performed by evacuation or by hysterotomy. In all of the 48 cases, the premedi-

1 Acknowledgment. The technical assistance of Miss Marjo Martonen is gratefully acknowledged. This investigation was supported by a grant from the National Research Council for Medical Science, Finland.

2 S. Aronson, G. Gennser, Ch. Owman and N.-O. Sjöberg, Eur. J. Pharmac. *11*, 178 (1970).
3 J. B. Read and G. Burnstock, Devl Biol. *22*, 513 (1970).
4 A. Hervonen, Acta physiol. scand. suppl. *368* (1971).
5 W. C. Dail and G. C. Palmer, Anat. Res. *177*, 265 (1973).
6 S. Partanen and O. Korkala, Experientia *30*, 798 (1974).
7 L. Kanerva, A. Hervonen and H. Hervonen, Med. Biol. *52*, 144 (1974).
8 G. Gennser and E. Nilsson, Experientia *26*, 1105 (1970).
9 D. Walker, Biol. Neonate *25*, 31 (1975).
10 S. Saarikoski, Acta physiol. scand. suppl. *421* (1974).