

MYOTROPIC EFFECTS OF NEW PROCTOLIN ANALOGUES MODIFIED IN THE POSITION 5 OF PEPTIDE CHAIN IN INSECTS

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To explain the role of the Thr⁵ residue of proctolin (Arg-Tyr-Leu-Pro-Thr) in the myotropic activity of this insect neuropeptide, we synthesized two groups of its analogues: 1) Arg-Tyr-Leu-Pro-X-OH with X = Val (**1**), D-Val (**2**), Ile (**3**), D-Ile (**4**), Ala (**5**), D-Ala (**6**), Asn (**7**), Gln (**8**), Ser (**9**), Pro (**10**), Phe (**11**), Asp (**12**), Glu (**13**), Arg (**14**), D-Arg (**15**), Lys (**16**) and Gly (**17**) and 2) Arg-Tyr-Leu-Pro-R', where R' = isobutylamine (**18**), S-1-methyl-1-phenylmethylamine (**19**), R-1-methyl-1-phenylmethylamine (**20**), R-2-amino-1-propanol (**21**), S-2-amino-1-propanol (**22**), R-1-amino-2-propanol (**23**), S-2-amino-1-propanol (**24**), 3-amino-1-propanol (**25**). Decapeptide proctolylproctolin (H-Arg-Tyr-Leu-Pro-Thr-Arg-Tyr-Leu-Pro-Thr-OH) (**26**) was synthesized. Syntheses of these peptides were carried out by solid-phase method. All peptides were bioassayed *in vitro* on the semi-isolated hearts of *Tenebrio molitor* using a cardioexcitatory test and on the foregut of locust (*Schistocerca gregaria*). Peptides **1**, **3**, **5**, **9**, **13**, **14**, **16**, **22**, and **23** retained about 30–50% of the cardioexcitatory activity in *T. molitor*. Analogues **1** and **3** preserved about 50% and analogue **8** about 80% of the myotropic activity, whereas compound **4** and **9** showed a very weak contractile activity in *S. gregaria*.

Key words: proctolin analogues, myotropic effect in insects, insect neuropeptide proctolin and analogues

Abbreviations: Boc – *tert*-butyloxycarbonyl, Bzl – *benzyl*, HOBt – *1*-hydroxybenzotriazole, Me – *methyl*, TFA – *trifluoroacetic acid*, Z – *benzyloxycarbonyl*

The symbols of the amino acids, peptides, and their derivatives are in accordance with the Recommendation of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (1984), Eur. J. Biochem. 1984, 138, 9.

INTRODUCTION

Proctolin, first structurally characterized myotropic insect neuromodulator, pentapeptide H-Arg-Tyr-Leu-Pro-Thr-OH, was isolated in 1975 from the whole body extracts of the American cockroach *Periplaneta americana* [1]. It was found later in six other orders of insects as well as in other invertebrates [5, 6]. Since the biological effect of proctolin consists in stimulation of contractions of smooth, skeletal, heart, and oviduct muscles of insects and other invertebrates [5, 6] it is considered as an insect neuromodulator.

In the course of structure-myotropic function studies on insects, a hundred of proctolin analogues were synthesized, of which twenty five retained agonistic effect in the selected insect species whereas about ten compounds showed antagonistic properties [2–5].

Encouraged by earlier investigations on proctolin we performed further studies to explain the role of the Thr⁵ residue in its myotropic activity. In earlier papers [5, 11] only four proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH) analogues modified in position 5, such as [Ala⁵]-, [D-Thr⁵]-, [Thr-NH₂⁵]- [11], and [N-Me-Thr⁵]-proctolin [2, 5] have been described. Among these analogues only two, [Ala⁵]- and [N-Me-Thr⁵]-proctolin, showed agonistic effect in relation to the oviduct of the locust *L. migratoria* [9] and the foregut of *S. gregaria* [2, 5], respectively. These data suggest that the role of the C-terminal part of the proctolin molecule needs further studies.

Basing on in our previous results of studies on structure/activity relationship in neuropeptide proctolin, we performed the synthesis of two groups of the C-terminally modified proctolin analogues: 1) proctolin analogues modified by L or D amino acid residues – H-Arg-Tyr-Leu-Pro-X-OH, where X = Val (1), D-Val (2), Ile (3), D-Ile (4), Ala (5), D-Ala (6), Asn (7), Gln (8), Ser (9), Pro (10), Phe

(11), Asp (12), Glu (13), Arg (14), D-Arg (15), Lys (16) and Gly (17) and 2) Arg-Tyr-Leu-Pro-R', where R' = isobutylamine (18), S-1-methyl-1-phenylmethylamine (19), R-1-methyl-1-phenylmethylamine (20), R-2-amino-1-propanol (21), S-2-amino-1-propanol (22), R-1-amino-2-propanol (23), S-2-amino-1-propanol (24), 3-amino-1-propanol (25).

The purpose of these studies was to explain the role of the C-terminal carboxyl group for proctolin myotropic properties in insects as well as the role of the structure of a side chain of amino acid residues at position 5 of the proctolin molecule. Moreover, decapeptide proctolyproctolin (H-Arg-Tyr-Leu-Pro-Thr-Arg-Tyr-Leu-Pro-Thr-OH) (26) was also synthesized.

Analogues 1–17 we obtained by substitution of the C-terminal Thr residue by L or D amino acid residues with the back side chains isosteric in relation to Thr, such as: Val (1) and Ile (3). Analogues 18–25 were obtained by the exchange of C-terminal Thr for amine derivatives. Proctolyproctolin (26) was designed by analogy to biologically active tetrapeptide tuftsin, an immunomodulating tetrapeptide with phagocytosis stimulating properties. The linear elongation of its peptide sequence to tuftsinyltuftsin gave an octapeptide with anticancer activity [8].

Syntheses of all peptides were carried out by the solid-phase method according to standard procedure. Biological activity of these peptides was evaluated by their myotropic activity in the *in vitro* heart preparation of *Tenebrio molitor* [10] and contractile activity in the isolated foregut of locust *S. gregaria* [2].

MATERIALS and METHODS

CHEMICAL PART

General methods

The amino acid composition was confirmed using a Mikrotechna T339M analyzer (Czechoslovakia). The optical activity of the chiral compounds was determined with a Polamat polarimeter (Carl Zeiss, Jena, Germany). The molecular weights of the peptides were determined using the Finigan Mat TSQ 700 (USA). TLC was performed in 3 solvent systems on silica gel and visualized using ninhydrin.

Synthesis methods

Proctolin was obtained from Sigma Chemical Co. Ltd. Peptides **1–26** were synthesized by the solid-phase method according to the Boc-procedure [5–7]. Dicyclohexylcarbodiimide (DCC) in the presence of 1-hydroxybenzotriazole (HOBt) was used as a coupling reagent. The C-terminal amino acids were bound to the Merrifield resin by a cesium salt procedure to give substitution level of 0.7 mMol/g of Boc-Y-OH, where Y = Val, D-Val, Ile, D-Ile, Ala, D-Ala, Asn, Gln, Ser(OBzl), Thr(OBzl), Pro, Phe, Asp, Arg(Tos), D-Arg(Tos), Lys(Z), Gly and Glu. The protected amino acids were coupled using DCC as a coupling reagent or by the linear anhydride methods. The following amino acid derivatives were used: Boc-Arg(Tos)-OH, tri-Z-Arg-OH, Boc-Tyr(OBzl)-OH, Boc-Leu-OH, Boc-Pro-OH, Boc-Thr(Bzl)-OH, Boc-Val-OH, Boc-D-Val-OH, Boc-Ile-OH, Boc-D-Ile-OH, Boc-Ala-OH, Boc-D-Ala-OH, Boc-Asn-OH, Boc-Gln-OH, Boc-Ser(OBzl)-OH, Boc-Lys(Z)-OH, Boc-Glu(Bzl)-OH, Boc-D-Arg(Tos)-OH, Boc-Phe-OH, Boc-Asp(Bzl)-OH and Boc-Gly-OH (Bachem). To synthesize of peptides **18–25**, the following amines were used, as: isobutylamine, S-1-methyl-1-phenylmethylamine, R-1-methyl-1-phenylmethylamine, R-2-amino-1-propanol, S-2-amino-1-propanol, R-1-amino-2-propanol, S-2-amino-1-propanol, 3-amino-1-propanol.

The amino acids and DCC were used in 3-fold excess. The N^α-Boc-group was subsequently removed with 30% TFA in dichloromethane (DCM) according to standard methods. The neutralization was made with 10% triethylamine (TEA) in DCM. Finally, peptides (**1–17** and **26**) were obtained by deprotection and cleavage from the support resin with trifluoromethanesulfonic acid (CF₃SO₃H) in anisole. Peptides (**18–25**) were cleaved from resin with 20% solution of abovementioned amines and then deprotected by catalytic hydrogenation in the presence of 10% Pd/C. All peptides were purified on Sephadex G-25 column by elution with 5% acetic acid and then by preparative HPLC. The physicochemical data were summarized in Table 1.

BIOASSAY PROCEDURES

Insects

Biological studies were performed on two insect species *in vitro*: cardioexcitatory action was

evaluated on the heart of *T. molitor* [10] and contractile and myotropic activity was investigated on the isolated foregut of locust *S. gregaria* [2]. Adult males of the yellow mealworm, *T. molitor*, were reared in the laboratory as described previously [10]. As the age of mealworm parents is of principal significance for the development and metabolism of their offspring, all insects used for bioassays came from parents which were mated when they were less than 1 month old. Adult locust (*S. gregaria*) of both sexes reared in laboratory culture (Blades Biological, Kent, UK) were used throughout this study, the colony was maintained at 27°C and fed on grass twice daily with a constant access to fresh water.

Cardioexcitatory assay

Peptides were bioassayed *in vitro* on semi-isolated heart preparations of *T. molitor* according to the method of Rosiński and Gäde [10]. Adult males (7-day-old) were decapitated and their abdomens were removed as close to the metathorax as possible. The ventral body wall of the abdomen was trimmed away so that lateral spiracular structures remained attached to the dorsal scleritis. The fat body, digestive organs, and Malpighian tubules were removed from the abdominal dorsum. It then contained the dorsal vessel, i.e. the heart, alary muscles, internal body muscles, the tracheae, and the dorsal cuticle. The heart preparations were selected on the basis of frequency (47–52 beats per minute) and regularity, and then they were superfused in *Tenebrio* saline (274 mM NaCl, 19 mM KCl, 9 mM CaCl₂, 5 mM glucose, and 5 mM HEPES, pH 7.0) until the stabilization was reached (20 min). Only regularly beating hearts were used in the assays. An open perfusion system was used, with the open point suspended 7 cm above the superfusion chamber. The heart was subjected to a constant perfusion of fresh saline at the rate of about 140 l/min. During bioassay, saline flowed directly from the open point to the perfusion chamber onto the caudal extremity of the heart. The saline flowed through the length of the heart and was removed by suction with a Whatman paper at the cephalic of the preparation. The activity of the heart could be observed through the transparent cuticle, and it was recorded automatically using a Microdensitometer MD-100 (Carl Zeiss, Jena, Germany). All tested samples were applied at the open point with a Hamilton syringe. Many pulse applica-

Table 1. Physicochemical data on free peptides

PEPTIDE	Yield (%)	Rt ^a (HPLC)	[α] _D ²⁰ c = 0.1 CH ₃ OH	TLC ^b Rf			AMINO ACID ANALYSIS	Mw	
				X	Y	Z		Calc.	Found
H-Arg-Tyr-Leu-Pro-Val-OH (1)	55	19.27	-36.4	0.36	0.71	0.64	Arg 1.0 Tyr 0.9 Leu 1.1 Pro 0.9 Val 1.0	646.9	647.6
H-Arg-Tyr-Leu-Pro-D-Val-OH (2)	72	25.26	-38.7	0.28	0.62	0.60	Arg 0.99 Tyr 1.03 Leu 1.0 Pro 0.98 Val 1.0	646.9	647.6
H-Arg-Tyr-Leu-Pro-Ile-OH (3)	67	21.83	-9.2	0.35	0.56	0.15	Arg 0.97 Tyr 0.99 Leu 1.0 Pro 1.03 Ile 1.0	660.8	661.8
H-Arg-Tyr-Leu-Pro-D-Ile-OH (4)	76	21.40	-29.1	0.31	0.46	0.24	Arg 1.0 Tyr 1.02 Leu 1.0 Pro 0.98 Ile 1.0	660.8	661.9
H-Arg-Tyr-Leu-Pro-Ala (5)-OH	44	14.44	-40.8	0.20	0.62	0.31	Arg 0.86 Tyr 0.80 Leu 0.99 Pro 1.25 Ala 1.1	618.7	619.7
H-Arg-Tyr-Leu-Pro-D-Ala-OH (6)	56	18.67	-33.7	0.28	0.70	0.37	Arg 1.0 Tyr 0.96 Leu 0.92 Pro 1.1 Ala 1.05	618.7	619.9
H-Arg-Tyr-Leu-Pro-Asn-OH (7)	52	15.62	-33.5	0.23	0.41	0.17	Arg 0.9 Tyr 0.99 Leu 0.97 Pro 1.1 Asp 1.05	661.9	662.8
H-Arg-Tyr-Leu-Pro-Gln-OH (8)	33	17.21	-18.9	0.43	0.57	0.34	Arg 0.89 Tyr 1.1 Leu 0.88 Pro 1.2 Glu 0.99	675.8	676.8
H-Arg-Tyr-Leu-Pro-Ser-OH (9)	46	18.45	-46.8	0.18	0.32	0.26	Arg 1.0 Tyr 0.90 Leu 0.90 Pro 1.1 Ser 1.1	633.8	634.5
H-Arg-Tyr-Leu-Pro-Pro-OH (10)	64	18.64	-43.3	0.20	0.60	0.35	Arg 1.1 Tyr 0.90 Leu 1.0 Pro 2.0	644.8	645.7
H-Arg-Tyr-Leu-Pro-Phe-OH (11)	54	12.86	-21.0	0.15	0.54	0.43	Arg 1.0 Tyr 0.90 Leu 1.0 Pro 1.0 Phe1.1	694.9	695.3
H-Arg-Tyr-Leu-Pro-Asp-OH (12)	41	18.26	+24.5	0.33	0.57	0.40	Arg 1.0 Tyr 10.97 Leu 1.03 Pro 1.0 Asp 1.0	662.8	663.6
H-Arg-Tyr-Leu-Pro-Glu-OH (13)	38	17.87	-28.9	0.42	0.52	0.38	Arg 1.0 Tyr 0.90 Leu 1.0 Pro 1.1 Glu 1.0	676.6	677.8
H-Arg-Tyr-Leu-Pro-Arg-OH (14)	42	18.78	-19.4	0.46	0.65	0.33	Arg 2.0 Tyr 1.0 Leu 0.80 Pro 1.2	703.6	704.8
H-Arg-Tyr-Leu-Pro-D-Arg-OH (15)	46	18.32	-16.4	0.35	0.27	0.12	Arg 1.96 Tyr 1.04 Leu 1.0 Pro 1.0	703.6	704.8
H-Arg-Tyr-Leu-Pro-Lys-OH (16)	71	17.06	-42.6	0.50	0.45	0.73	Arg 1.0 Tyr 1.0 Leu 1.2 Pro 0.8 Lys 1.0	675.7	676.9
H-Arg-Tyr-Leu-Pro-Gly-OH (17)	72	22.17	-42.2	0.17	0.56	0.27	Arg 0.9 Tyr 0.98 Leu 1.08 Pro 1.0 Gly 1.04	605.7	606.2
H-Arg-Tyr-Leu-Pro-NHCH ₂ CH(CH ₃) ₂ (18)	51	22.56	-31.9	0.35	0.56	0.15	Arg 0.95 Tyr 1.05 Leu 1.0 Pro 1.0	602.8	603.5
H-Arg-Tyr-Leu-Pro-(S)-NHCH(CH ₃)C ₆ H ₅ (19)	42	18.62	-44.2	0.27	0.34	0.30	Arg 1.0 Tyr 0.96 Leu 1.02 Pro 1.02	650.8	651.8
H-Arg-Tyr-Leu-Pro-(R)-NHCH(CH ₃)C ₆ H ₅ (20)	44	19.86	-29.6	0.24	0.19	0.35	Arg 0.92 Tyr 1.0 Leu 1.06 Pro 1.0	650.8	651.7
H-Arg-Tyr-Leu-Pro-(R)-NHCH(CH ₃)CH ₂ OH (21)	43	20.76	-30.6	0.34	0.61	0.65	Arg 0.92 Tyr 1.04 Leu 1.02 Pro 1.0	604.6	605.4
H-Arg-Tyr-Leu-Pro-(S)-NHCH(CH ₃)CH ₂ OH (22)	39	23.45	+23.8	0.36	0.52	0.45	Arg 1.0 Tyr 0.98 Leu 1.02 Pro 1.0	604.6	605.3

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Table 1. continued from previous page

PEPTIDE	Yield (%)	Rt ^a (HPLC)	[α] _D ²⁰ c = 0.1 CH ₃ OH	TLC ^b Rf			AMINO ACID ANALYSIS	Mw	
				X	Y	Z		Calc.	Found
H-Arg-Tyr-Leu-Pro-(R)-NHCH ₂ CH(OH)CH ₃ (23)	42	19.75	-15.8	0.40	0.76	0.58	Arg 0.94 Tyr 0.99 Leu 1.02 Pro 1.05	604.6	605.8
H-Arg-Tyr-Leu-Pro-(S)-NHCH ₂ CH(OH)CH ₃ (24)	33	17.43	-29.7	0.48	0.34	0.17	Arg 0.99 Tyr 1.02 Leu 1.02 Pro 0.97	604.6	605.9
H-Arg-Tyr-Leu-Pro-NHCH ₂ CH ₂ CH ₂ OH (25)	46	21.33	-23.7	0.22	0.27	0.32	Arg 1.12 Tyr 1.0 Leu 0.90 Pro 0.98	604.7	605.8
H-Arg-Tyr-Leu-Pro-Thr-Arg-Tyr-Leu-Pro-Thr-OH (26)	48	17.21	-18.9	0.26	0.42	0.45	Arg 2.0 Tyr 2.14 Leu 2.0 Pro 1.86 Thr 2.00	1278.6	1279.2

^a HPLC on Ultrasphere ODS column (Beckman) 4.5 × 250 mm; gradient: 0–80% solvent B for 60 min (B = 80% acetonitrile in water + 0.1% TFA), detected at wavelength 222 nm ^b TLC on silica gel plates (Merck) eluents: X = n-butanol : Ac-OH : water (4:1:5), Y = n-butanol : pyridine : Ac-OH : water (30:20:6:24), Z = n-butanol : Ac-OH : water (4:1:1)

tions of samples could be sequentially assayed on a single preparation. The open system was designed to enable samples to be added without causing a change in pressure. All assays were performed at room temperature (21 ± 1°C). The stimulatory activities of the tested peptides are presented as an increase in the heartbeat frequency. The dose-response relationship was established for each proctolin analogue (separate determinations for 6–10 insects ± SEM).

Contractile activity on the foregut

Isolated foreguts of locust *S. gregaria* (oesophagus to proventriculus) [2] were incubated in Clarke Insect Ringer (NaCl: 113.7mM; KCl: 1.9 mM; CaCl₂: 1.1 mM; NaHCO₃: 0.12 mM; Na₂PO₄: 0.07 mM; pH 6.8) at room temperature (18 ± 2°C) for 20 min prior to testing the contractile effects of proctolin and a range of its pentapeptide analogues. All peptides were reconstituted in water, and subsequently working solutions were prepared using Clarke Insect Ringer. Ligatures were placed at the oesophageal and proventricular regions of the foregut prior to isotonic recording of tissue length. The oesophageal ligature was connected to a Washington Instruments type T2 isotonic transducer whose output was fed into Washington Instruments MD2R-ink writing oscillograph via a FC117 coupler while the proventricular ligature was attached to a glass rod in a 5 ml vertical organ bath. The contents of the bath were aerated constantly and could be replaced rapidly. The tissue was stimulated using a 6 min cycle with two washes and

dose-response curves were constructed over the concentration range 10⁻⁹–10⁻⁶ M, for proctolin and analogues **1–26**. Agonists were allowed to remain in contact with the tissues for 2 min prior to being washed off. Tissues were washed a second time for 4 min in each cycle. When testing peptides with no obvious agonist action for potential antagonistic properties, the putative antagonist was added to the tissue 30 s before adding the next dose of proctolin.

Data analysis

Bioassay data are shown as means of 8 replicates (± SEM). Dose-response curves were constructed for proctolin and its analogues by expressing tissue contraction as a percentage of the maximum proctolin response, using Fig P for Windows (Biosoft) running on an IBM 486 D X 2 PC. The data presented for analogues are paired, i.e. each control was performed on the same day as the test using the same tissue, and are the means of experiments carried out on 6–8 different tissues. Statistical significance was assessed by use of Student's *t*-test; *p* values of 0.05 or less were considered significant. Calculations were performed using the computer programme 1-2-3 release 5 for Windows (Lotus).

RESULTS and DISCUSSION

Peptides **1, 3, 5, 9, 13, 14, 16, 22** and **23** in *T. molitor* retained about 30–50% of the proctolin activity (Fig. 1). Other peptides including proctolyl-proctolin (**26**) were practically inactive.

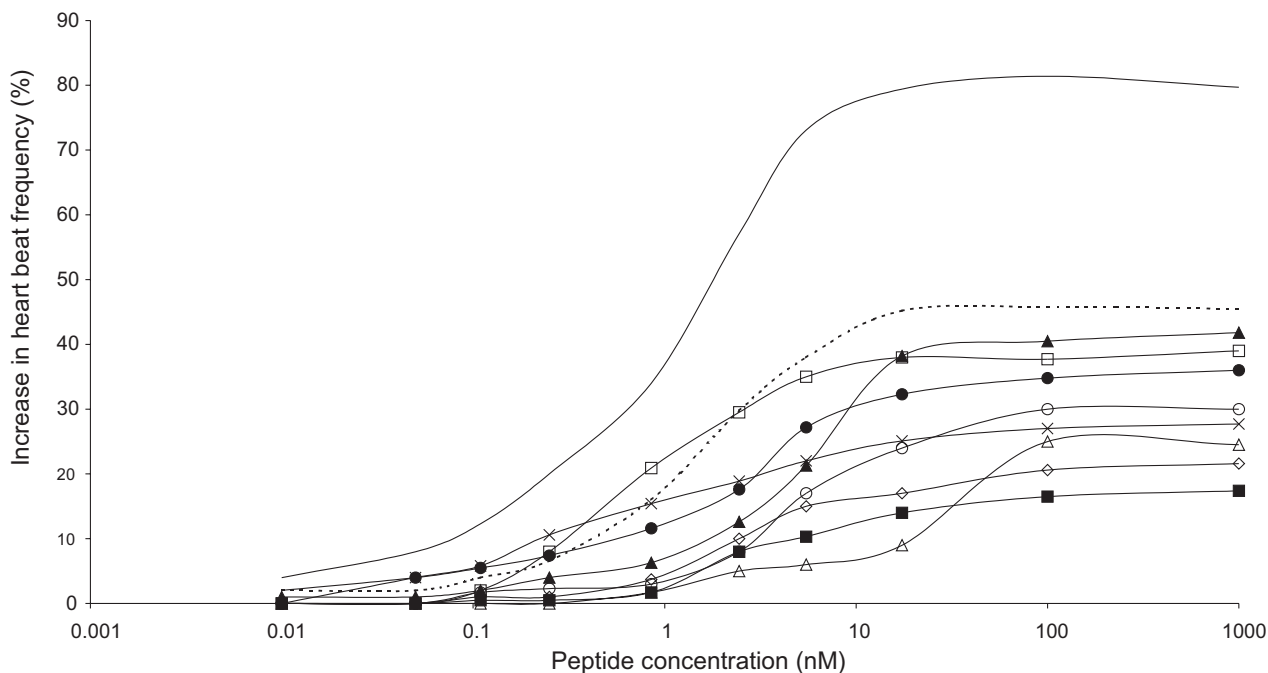


Fig. 1. Cardioexcitatory effect of proctolin and its analogues in *Tenebrio molitor* ($6-10 \pm \text{SEM}$) — proctolin, - - - Arg-Tyr-Leu-Pro-Val (1), ▲ Arg-Tyr-Leu-Pro-Ile (3), ● Arg-Tyr-Leu-Pro-Ala (5), △ Arg-Tyr-Leu-Pro-Ser (9), ○ Arg-Tyr-Leu-Pro-Glu (13), ◇ Arg-Tyr-Leu-Pro-Arg (14), ■ Arg-Tyr-Leu-Pro-Lys (16), □ Arg-Tyr-Leu-Pro-(S)-NH CH(CH₃)CH₂OH (22), × Arg-Tyr-Leu-Pro-(R)-NH CH₂CH(OH)CH₃ (23)

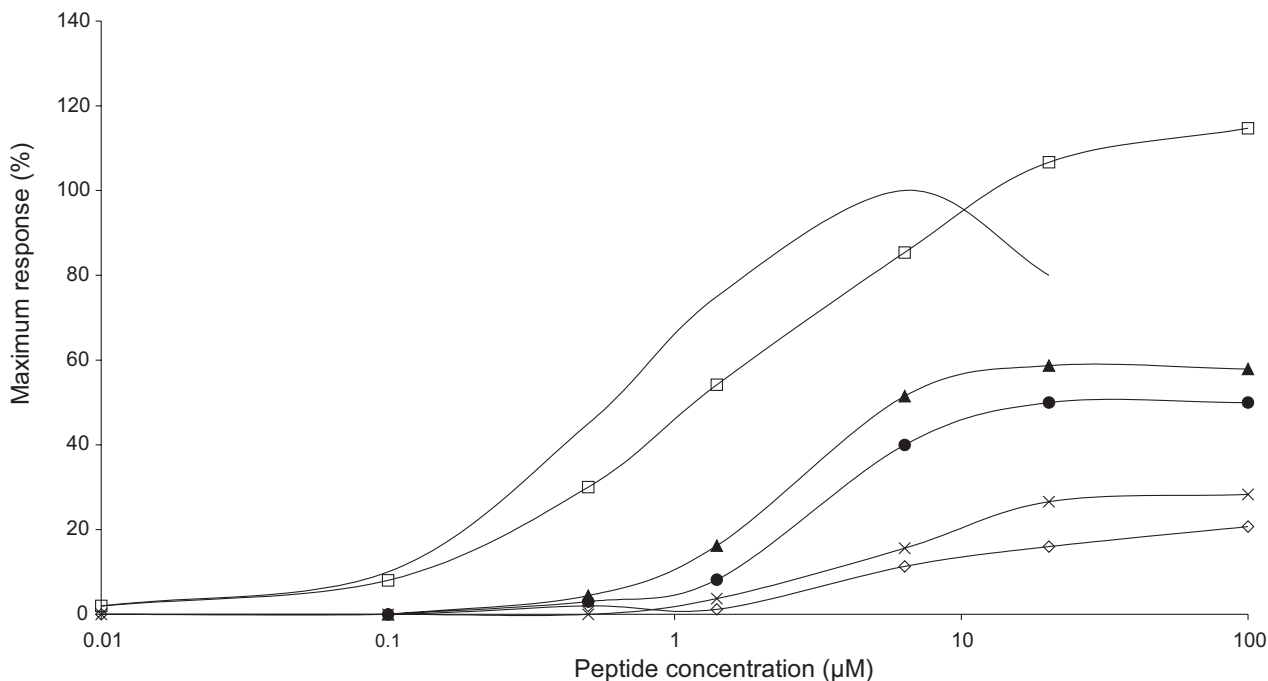


Fig. 2. Myotropic effect of proctolin and its analogues on the foregut of *Schistocerca gregaria* ($8 \pm \text{SEM}$) — proctolin, ▲ Arg-Tyr-Leu-Pro-Val (1), ● Arg-Tyr-Leu-Pro-Ile (3), × Arg-Tyr-Leu-Pro-D-Ile (4), □ Arg-Tyr-Leu-Pro-Gln (8), ◇ Arg-Tyr-Leu-Pro-Ser (9)

We found that analogues **1**, **3**, and **8** retained about 50–80% of the proctolin-like activity in myotropic test on the foregut of *S. gregaria* (Fig. 2) whereas peptides **4** and **9** had weak stimulatory properties in the test evaluating contraction of the locust foregut on peptide concentrations ranging from 10^{-7} to 10^{-6} M. Other peptides had neither agonistic nor antagonistic activity.

It is interesting that analogues **1** and **3**, containing Val or Ile with the isosteric side chain in relation to Thr, stimulated the heart-beat frequency in *T. molitor* and contraction of the *S. gregaria* foregut. It testifies that the presence of methyl or ethyl groups isosteric with the threonine side chain is sufficient for preservation of the myotropic effect in insects. In addition, it should be pointed out that proctolin analogue **22**, containing S-2-amino-1-propanol in position 5, and analogue **23**, containing R-1-amino-2-propanol with the side chain isosteric in relation to side chain of Thr, retained about 50% proctolin myotropic activity.

Moreover, the species specificity was observed in the case of [D-Ile⁵]- (**4**) and [Gln⁵]-proctolin (**8**). These different myotropic effects, observed in two insects, depend probably on the structural requirement for the specific amino acid residue in position 5 of proctolin molecule in both tested insects. For instance, a peptide modified in position 5 of the proctolin chain by Ala (analogue **5**) weakly stimulated the heart of yellow mealworm. The same analogue (**5**) showed no stimulatory effect in the test performed on the foregut of *S. gregaria*. Similar species specificity was observed in the case of analogues **4** and **8**, which contained D-Ile or Gln instead of Thr-5. These analogues preserved 30% and 80% of the proctolin activity in locust, respectively, while lacked this activity in the yellow mealworm. The different response of the investigated peptides in two insect species is probably a consequence of structurally different requirement of receptors in the *T. molitor* heart and *S. gregaria* foregut.

CONCLUSIONS

The following conclusions can be drawn from analysis of the myotropic effects of proctolin analogues (**1–26**) on two preparations.

- 1) These results point out that the presence of the C-terminal Thr residue plays an important role in myotropic activity of proctolin in insects;
- 2) The presence of amino acid residues with the side chain isosteric in relation to Thr in the position 5 of proctolin is sufficient for of the myostimulatory effect of the investigated substances;
- 3) The elongation of the proctolin sequence to decapeptide (proctolyproctolin, analogue **26**) yields analogues without myotropic activity in insects;
- 4) Species specificity plays an important role in the evaluation of the biological activity of newly synthesized proctolin analogues;
- 5) The lack of activity of analogues containing amines instead of Thr in position 5, except for analogues **22** and **23** which retained 40–50% of the proctolin activity, testifies that the presence of the carboxyl group in the C-terminal amino acid as well as of the -OH group in the side chain plays an essential role in myotropic properties in insects.

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