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Central activity of peptide Gly-Pro-Hyp – the main component of collagen degradation products mixture

Konstanty Wiśniewski, Barbara Artemowicz, Anna Lutostańska, Józef Maćkowiak and **Wiktor Koziołkiewicz**[1]

Department of Pharmacology, Medical Academy, 2c Mickiewicz St., 15-222 Białystok; [1]Departament of Physiology and Biochemistry, Medical Academy, Łódź, Poland

Abstract. The effect of tripeptide Gly-Pro-Hyp on the central nervous system was studied. The peptide Gly-Pro-Hyp is a main component of the mixture of collagen type I degradation products obtained by 6-h digestion with bacterial collagenase. The investigated peptide in the doses 1 μg, 2 μg, and 5 μg significantly reduces the psychomotor activity of animals, intensifies haloperidol-induced catalepsy and enhances stereotypy behaviour after apomorphine administration. The central activity of Gly-Pro-Hyp is similar to the action of collagen degradation products mixture. This suggests that this peptide may be the active substance responsible for the effects of degradation products of collagen type I.

Address for correspondence:
Konstanty Wiśniewski
Department of Pharmacology
Medical Academy
2c Mickiewicz St.,
15-222 Białystok, Poland

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INTRODUCTION

Collagen is the most abundant animal protein in mammals, accounting for about 30% of all proteins. It is present in connective tissue and contributes to the structural framework of most organs. In human tissues 13 different collagen types have been identified so far, but collagen type I constitutes between 80% and 99% of the total collagen (Burgeson et al. 1992). It is composed of two identical- alpha 1 chains and one different- alpha 2 chain (trihelical structure). The most frequent amino acid sequence in the trihelical fragments is Gly-Pro-X. The most frequent amino acid in the X position is Hyp. Collagen is degraded by enzymes- bacterial and tissue collagenases. The peptide Gly-Pro-Hyp makes up about 36% of the molecular weight of the collagen degradation products mixture obtained after 6 hours of digestion of trihelical fragments of collagen type I with bacterial collagenase (Telejko et al. 1986, 1990, Wróbel 1992). Previous studies have shown that degradation products of trihelical fragments of collagen ($DPTC_6$) are biologically active substances and exert an effect on the central nervous system (CNS) (Buczko et al. 1980, Telejko et al. 1986, 1990, 1992). A whole mixture of $DPTC_6$ reduces the psychomotor activity of rats as evaluated by the Lat's test and intensifies the haloperidol-induced catalepsy. $DPTC_6$ given immediately before apomorphine enhances the stereotypy behaviour of rats, but did not evoke significant effect on amphetamine-induced stereotypy. It has been found that $DPTC_6$ can undergo further degradation (Wróbel 1992) and its action may depend on shorter fragments, perhaps the tripeptide Gly-Pro-Hyp.

The purpose of our study was to estimate the activity of the tripeptide Gly-Pro-Hyp, especially its central action.

METHODS

The peptide Gly-Pro-Hyp was obtained on synthetic way by classical method in solution. (W. Koziołkiewicz, Departament of Physiology and Biochemistry, Medical Academy, Łódź). Homogenity of the peptide was conformed by several chromatography methods.

All experiments were performed on 240 male Wistar rats with a mean body weight of 182,9g (SD 8,3). The animals were kept in a room with a light-dark cycle of 12 : 12h . The studies were performed at the same hours, between 8^{00} and 14^{00}, every day. The peptide was dissolved in physiological saline and injected into the lateral cerebral ventricule (i.c.v.) of the rats in a volume of 10 µl by means of a Hamilton microsyringe according to the method of Herman (1970).

Three doses of peptide (1 µg, 2 µg, 5 µg) were used. They were established adequately to their percentage contents (36,2%) in previously used doses of $DPTC_6$ (2,5 µg, 5 µg and 15 µg). The doses of $DPTC_6$ empirically established were most effective in previous studies. (Telejko et al. 1990). The control group received 0.9% NaCl in a volume 10 µl i.c.v.

For estimation of the effect of tripeptide on CNS same behavioural tests were used.

The psychomotor activity of rats was evaluated by the test of Lat (1965). The animals were observed for 10 min before injection of tripeptide. The number of seconds of walking, immobility, washing and standing-up behaviours were recorded. These measures were admited as 100%. The behaviour of the rats was observed again 30 min after i.c.v. administration of the Gly-Pro-Hyp. The same behaviours were recorded again.The results were submited in percentages in comparison to previous observation.

Stereotypic behaviour of animals was rated according to a scale described by Kennedy and Zigmond (1978): -1 quiet or asleep; 0 normal activity; 1 occasional non-directed sniffing; 2 continuous sniffing; 3 continuous sniffing on a restricted area of the floor; 4 as 3 but with occasional licking; 5 continuous licking; 6 continuous licking with occasional biting; 7 continuous biting. Stereotypy was induced by an intraperitoneal (i.p.) injection of apomorphine (APO; Sandoz; 2 mg/kg) or amphetamine (AMPH; Warsaw, Pharmaceutical Laboratories,

Polfa, 6.5 mg/kg i.p.). Gly-Pro-Hyp was introduced i.c.v. immediately before application the stereotypy-inducing drugs.

Catalepsy was evaluated by the method of Simon et al. (1968) slightly modified (Braszko et al. 1993) and was evoked by haloperidol (HAL; Richter, Budapest). Six tests were applied in the followig order: 3 cm high rod, parallel bars, four corks, crossings of both limbs on either side of the trunk and sitting on the hind limbs and tail with both forelimbs withdrawn from the floor. Each test was scored 0 or 1. In the tests 1-4 score one was given if the rat stayed in the abnormal position for at least10 s, and in the tests 6 and 7 for 3 s. The scores of 6 tests were added so that the maximum score for one rat at the given time was 6. Haloperidol was applied i.p. in a dose of 0.5 mg/kg 30 min before the i.c.v. injection of Gly-Pro-Hyp.

The duration of thiopental- induced sleep was measured from the loss of postural reflexes to the recovery of the standing position. Thiopental (Roztoky, Praque) was given subcutaneously (s.c.) in a dose of 30 mg/kg 15 min after the peptide i.c.v. administration.

The influence of tripeptide on the latency and duration of cardiasol-produced seizures in rats was tested. Cardiasol (Penthylenotetrazole crystalline, Sigma) was applied s.c. in a dose of 75 mg/kg 15 min after Gly-Pro-Hyp injection.

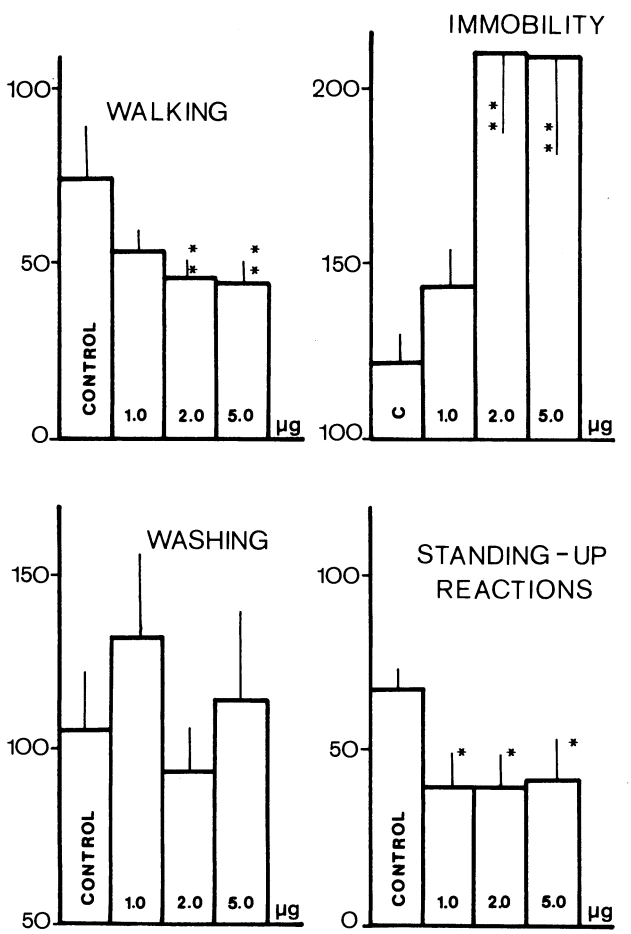

Fig. 1. The effect of various doses of Gly-Pro-Hyp (i.c.v. 30 min before observation) on psychomotor activity of rats in Lat's test. Each bar represent mean of 10 results ±SD; *P<0.05; ** P<0.01; as compared with the control (ANOVA and Newman-Keuls test).

Statistical analysis

The results of the experiments were evaluated by analysis of variance (ANOVA) followed by Newman-Keuls test. F-rations, degrees of freedom and P-values are reported only for significant differences. In all comparisons between particular groups a probability of 0.05 was considered significant. All ratings of particular tests for each rat were summed up first, and overall group means were then calculated.

RESULTS

The effect of Gly-Pro-Hyp on psychomotor activity

Gly-Pro-Hyp significantly reduced psychomotor activity of rats evaluated by Lat's test. ANOVA revealed significant differences between the groups. The time of walking was diminished ($F_{2,84} = 5{,}271$, P<0.01). The time of immobility was prolonged ($F_{2,84} = 5{,}699$, P<0.05). Gly-Pro-Hyp also reduced the time of washing and standing-up behaviour, but ANOVA not revealed significant differences between groups. The effect of doses 2 µg and 5 µg was the strongest. (Fig 1.).

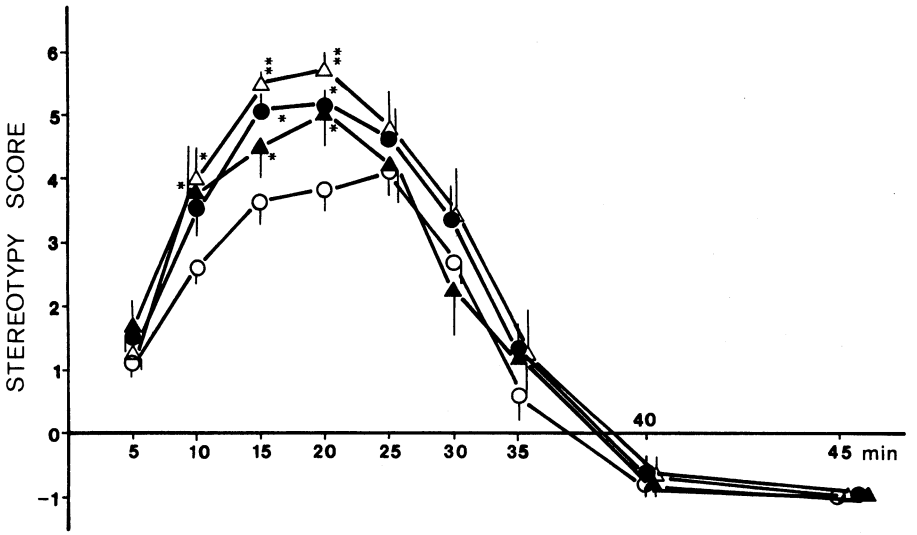

Fig. 2. The effect of various doses of Gly-Pro Hyp on the apomorphine- induced stereotypy behaviour in rats (Gly-Pro-Hyp i.c.v. immediately before stereotypy- induced drug). Open circles, control (APO 2 mg/kg i.p. + saline i.c.v.); open triangles, (APO + 1 μg of Gly-Pro-Hyp); filled circles, (APO + 2 μg of Gly-Pro-Hyp); filled triangles, (APO + 5 μg of Gly-Pro-Hyp). Each point is the mean of 10 results ±SD (ANOVA and Newman-Keuls test).

The effect of Gly-Pro-Hyp on stereotypy behaviour

Tripeptide Gly-Pro-Hyp administrated immediately before apomorphine intensified apomorphine-induced stereotypy behaviour of rats from 10th min to 20th min of observation.

For cumulative ratings of apomorphine stereotypy ANOVA yielded $F_{3,68}$=1,941. A subsequent analysis of differences with Newman-Keuls test did not revealed significant differences between groups.(Fig.2.). The peptide does not change amphetamine-induced stereotypy (results not shown).

The effect of Gly-Pro-Hyp on catalepsy

Gly-Pro-Hyp given i.c.v. 30 min before haloperidol injection considerably enhanced the effect of the cataleptic inducing drug. For cumulative ratings of catalepsy ANOVA revealed $F_{3,68}$=9,236. Further comparisons with Newman-Keuls test showed statistical significances: $P<0.01$ in case of doses of 1 μg and

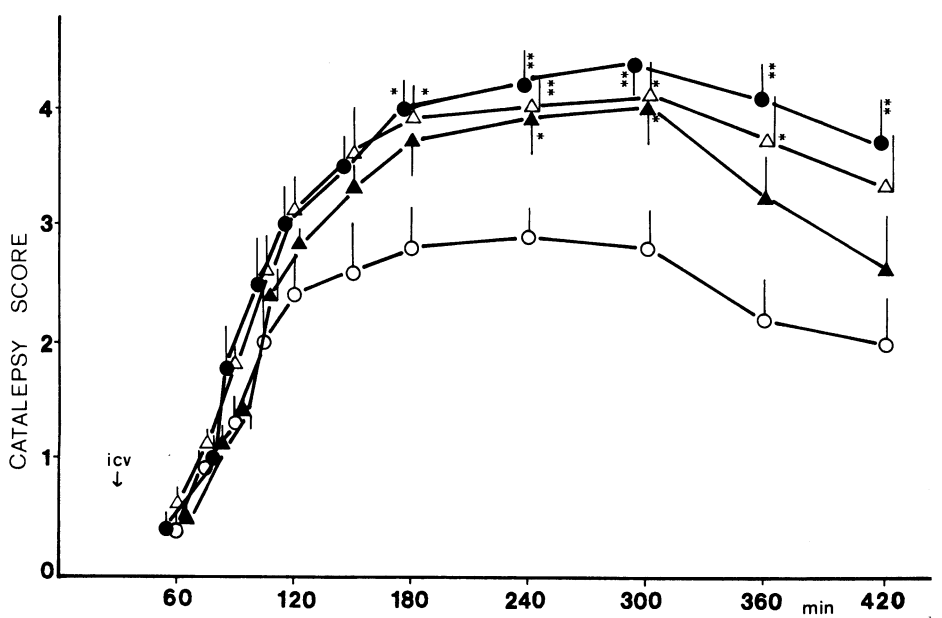

Fig. 3. The effect of various doses of Gly-Pro-Hyp on the haloperidol- induced catalepsy in rats (Gly-Pro-Hyp i.c.v. 30 min after HAL 0.5 mg/kg i.p.). Open circles, control (HAL + saline); open triangles, (HAL + 1 μg of Gly-Pro-Hyp); filled circles, (HAL + 2 μg of Gly-Pro-Hyp); filled triangles, (HAL + 5 μg of Gly-Pro-Hyp). Each point is the mean of 10 results ±SD. Overall effects of Gly-Pro-Hyp were significant. $F_{3,68}$=9,236; $P<0.05$ - 5 μg of Gly-Pro-Hyp; $P<0.01$ - 1 μg and 2 μg of Gly-Pro-Hyp (ANOVA and Newman-Keuls test).

2 μg and $P<0.05$ in case of dose of 5 μg of Gly-Pro-Hyp as compared with the control group (Fig.3.).

The effect of Gly-Pro-Hyp on the time of thiopental-induced sleeping

The investigated peptide did not influence sleeping time following thiopental administration in each dose (1 μg, 2 μg, and 5 μg) (results not shown)

The effect of Gly Pro-Hyp on the latency and duration of seizures produced by cardiasol

The seizures latency and duration were not changed by any dose of Gly-Pro-Hyp. ANOVA did not revealed differences between groups (Table I).

TABLE I

Effect of Gly-Pro-Hyp on the latency and duration of seizures produced by cardiasol

Drug	Seizures latency	Seizures duration
0.9% NaCl	579.6±457.2	27.9±23.1
	SE 99.8	SE 5.0
1 μg Gly-Pro-Hyp	387.7±186.0	29.0±21.9
	SE 56.1	SE 6.6
2 μg Gly-Pro-Hyp	378.1±139.4	27.8±13.4
	SE 42.0	SE 4.0
5 μg Gly-Pro-Hyp	442.8±263.0	28.1±18.5
	SE 79.3	SE 5.6

DISCUSSION

These results indicate that the tripeptide Gly-Pro-Hyp is a pharmacologically active substance and has a central activity. It reduces mobility and increases immobility of animals as evaluated by Lat's test. It also increases the cataleptic action of haloperidol. This effect may be connected with the low mobility of rats which was demonstrated in Lat's test. The tripeptide exerts some contradictory actions, such as intensification of the actions of agonists and antagonists of dopaminergic receptors (apomorphine and haloperidol). It is very intriguing that the tripeptide enhances apomorphine-induced stereotypy, but does not influences amphethamine-induced stereotypy. It seems that this effect may depend on the different mechanisms of actions of apomorphine and amphethamine.

The mechanism of action of Gly-Pro-Hyp is complex and difficult to explanation. Perhaps it does not act directly on the dopaminergic system, but indirectly by stimulation or inhibition of other neurotransmitters pathways. Data from previous studies show that products of proteolysis can play the role of modulators in the C.N.S. (Sobaniec et al. 1975, Gajner 1976, Wawrzyniuk et al. 1979, Wiśniewski 1979, Świderska et al.1986). A whole mixture of collagen degradation products and single amino acid characteristic for collagen - hydroxyproline act similary (Telejko et al. 1990, 1992, Lutostańska et al.1993).

Althought the Gly-Pro-Hyp bonds are resistant to the action of proteolytic enzymes (Wróbel 1992), it is possible, that in some conditions a part of bonds can undergo hydrolysis and the single amino acids may be active. The pharmacological activity of glycine is known (Zawadzka-Szeremeta 1979). This amino acid decreases the psychomotor activity of rats in Lat's test, enhances the apomorphine- and amphethamine-induced stereotypy and decreases catalepsy. We have shown that hydroxyproline is also a centrally active amino acid. (Lutostańska et al. 1993).

It is interesting that effects of Gly-Pro-Hyp in the C.N.S. do not depend on its doses in particular tests.

The results of previous studies suggest that collagen degradation products are biologically active substances (Buczko et al. 1980, Telejko et al. 1990, 1992). The tripeptide Gly-Pro-Hyp is an active component of this mixture.

The similarities between the actions of $DPTC_6$ and Gly-Pro-Hyp point to a participation of Gly-Pro-Hyp in the central activity of $DPTC_6$.

An increase in collagen degradation appears in some physiological and pathological states (growth, burns,rheumatic diseases) (Smiley et al. 1964, Ziff et al. 1965, Macek et al. 1987). The excretion of Hyp with urine (Hyp is a marker of col-

lagen degradation) is increased 20 times in patients with mental deficiency with hydroxyprolinemia, which is connected with disorders in hydroxyproline metabolism. (Smiley et al. 1964). This pathological state is seems remote from the metabolism of collagen in the organism.

The results of our studies shown that following pathologically accelerated collagen degradation the shorter fragments are released and are active in the CNS.

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