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Effect of *N*-3-(4-Hydroxyphenyl)propionyl Pro–Pro–Gly–Ala–Gly on Calcium-Induced Arrhythmias

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The present investigation was done to examine whether or not the presence of hydroxyproline in *N*-3-(4-hydroxyphenyl)propionyl Pro–Hyp–Gly–Ala–Gly (HP-5) is essential for the antiarrhythmic activity. Pretreatment of mice with 10 mg/kg of [Pro^2]-HP-5 provided significantly better protection against calcium-induced arrhythmias than did pretreatment with HP-5. Thus, the prolyl residue was more favorable than the hydroxyprolyl residue for antiarrhythmic activity of these analogues.

Keywords——peptide; antiarrhythmic effect; calcium; hydroxyproline; proline

A hexapeptide (antiarrhythmic peptide, AAP) isolated from bovine atria[1] and identified as Gly–Pro–Hyp–Gly–Ala–Gly[2] showed a protective effect against experimental drug-induced arrhythmias.[3] Several investigations[1,4] on the structure–activity relationship of AAP analogues revealed that *N*-3-(4-hydroxyphenyl)propionyl Pro–Hyp–Gly–Ala–Gly (HP-5) was the most potent inhibitor of calcium-induced arrhythmias. The peptide contains the unique amino acid hydroxyproline, which lacks a corresponding deoxyribonucleic acid (DNA) codon and is derived from proline by hydroxylation.[5] In this paper, a new analogue, [Pro^2]-HP-5, which contains proline instead of hydroxyproline, was prepared and its activity on calcium-induced arrhythmias was evaluated.

Experimental

Peptides——HP-5 prepared in our laboratory[4] was used in this experiment. [Pro^2]-HP-5 was synthesized by using dicyclohexylcarbodiimide as a condensing agent.[6] Briefly, 3-(4-benzyloxyphenyl)propionic acid was coupled with $(Pro)_2$–Gly–Ala–Gly·OBzl, and protecting groups were removed by catalytic reduction to give *N*-3-(4-hydroxyphenyl)propionyl Pro–Pro–Gly–Ala–Gly ([Pro^2]-HP-5). ^{1}H-NMR (in dimethylsulfoxide-d_6 with tetramethylsilane as an internal standard) δ: 1.15—1.3 (2H, m), 1.7—2.2 (6H, m), 2.45—2.55 (2H, m), 2.55—2.75 (2H, m), 3.2—3.65 (6H, m), 3.71 (2H, br), 4.2—4.4 (2H, m), 4.4—4.7 (1H, m), 6.66 (2H, d, J=8 Hz), 6.9—7.1 (2H, m), 7.55—7.65 (1H, m), 7.9—8.1 (1H, m), 8.25—8.4 (1H, m), 9.40 (1H, br). IR (KBr): 3300, 1620 cm^{-1}.

Measurement of Antiarrhythmic Activity——Test samples dissolved in saline and adjusted to pH 7 were administered intravenously. Verapamil hydrochloride, propranolol hydrochloride, disopyramide phosphate and lidocaine (products of Sigma Chemical Co., Mo., U.S.A.) were used as reference drugs. According to the method of Lynch *et al.*,[7] male ddY mice weighing 25—30 g were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and given continuously $CaCl_2$ (200 mg/kg/min, 0.15 ml/min) through the tail vein by slow infusion. The infusion was maintained until the animals died. Test samples were given 3 min before the $CaCl_2$ infusion started. The electrocardiogram (ECG), lead II, was continuously monitored on a Polygraph system RM-6200 (Nihon Koden). The time lags to the onset of arrhythmias were measured and compared to those from animals pretreated with saline (control group) and test samples. Arrhythmias were predominantly composed of an atrioventricular block (2nd or 3rd degree), and ventricular fibrillation.

TABLE I. Effect of HP-5 and [Pro^2]-HP-5 on Calcium-Induced Arrhythmias

Sample[a]	Dose (mg/kg)	No. of mice	Onset of arrhythmias[b] (s, Mean ± S.E.)
Control	—	43	147 ± 8
HP-5	10	22	180 ± 13[c]
[Pro^2]-HP-5	10	22	216 ± 12[d,e]
Verapamil	5	10	229 ± 27[d]
Propranolol	1	10	184 ± 15[c]
Disopyramide	10	10	164 ± 11
Lidocaine	10	10	163 ± 13

a) Sample or saline (control) was administered intravenously 3 min before the start of $CaCl_2$ infusion (200 mg/kg/min). *b*) The onset time of arrhythmias induced by $CaCl_2$ infusion was measured. See Experimental for details. *c*) $p<0.05$, *d*) $p<0.01$; *versus* control. *e*) $p<0.05$; *versus* HP-5.

Results and Discussion

Previously, we reported that 3-(4-hydroxyphenyl)propionylation of the imino nitrogen of proline in Pro–Hyp–Gly–Ala–Gly (P-5) led to increased potency. The present investigation was done to discover whether or not the presence of hydroxyproline in the peptide portion of HP-5 is necessary for the activity.

As shown in Table I, pretreatment of mice with 10 mg/kg of [Pro^2]-HP-5 provided significant protection against calcium-induced arrhythmias ($p<0.01$). Further, the potency of [Pro^2]-HP-5 was significantly higher than that of HP-5 ($p<0.05$). It is clear that the prolyl residue was more favorable than the hydroxyprolyl residue for antiarrhythmic activity of these analogues.

Hydroxyproline has been found only in proteins such as collagen and others containing collagen-like sequences such as complement C_{1q},[8] 18S acetylcholinesterase[9] and pulmonary glycoprotein.[10] Little is known about the occurrence of hydroxyproline-containing peptides of small molecular size in the animal kingdom, except for cleavage products of collagen, which exist in the serum and urine of mammals. Recently, [Hyp^3]-Lys-bradykinin was isolated from the kallikrein hydrolysate of plasma protein, besides Lys-bradykinin.[11] Presumably a peptide, Gly–Pro–Pro–Gly–Ala–Gly, might be present as a preform of AAP and might be more important physiologically than AAP. However, no evidence for this is yet available.

It is generally thought that excess $CaCl_2$ causes arrhythmias, mainly *via* the Ca-induced Ca release from sarcoplasmic reticulum and generation of a transient inward current.[12] In this mouse model, verapamil and propranolol showed antiarrhythmic activity, but disopyramide and lidocaine did not. It is noteworthy that the peptide analogues had no intrinsic effects on heart rate, blood pressure and ECG (data not shown), differing from verapamil[7] and propranolol.[13]

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