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STRUCTURE-ACTIVITY RELATIONSHIPS OF N-β-PHENYLPROPIONYL-L-TYROSINE AND ITS DERIVATIVES ON THE INHIBITION OF AN IDENTIFIABLE GIANT NEURONE OF AN AFRICAN GIANT SNAIL (*Achatina fulica* Férussac)

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1 Inhibitory effects of N-β-phenylpropionyl-L-tyrosine, N-β-phenylpropionyl-L-tryptophan and their derivatives on an identifiable giant neurone, TAN (tonically autoactive neurone) of an African giant snail (*Achatina fulica* Férussac) were examined in an attempt to elucidate which structural features are necessary to produce the effect.

2 Of the compounds examined, N-β-cyclohexylpropionyl-L-tyrosine showed the strongest effect. Its critical concentration (c.c.) was 3×10^{-8}–10^{-7} M, about ten times lower than that of N-β-phenylpropionyl-L-tyrosine (c.c., 3×10^{-7}–10^{-6} M). N-β-cyclohexylpropionyl-L-tryptophan (c.c., 10^{-6} M) had an effect almost similar to that of N-β-phenylpropionyl-L-tryptophan (c.c., 10^{-6} M).

3 N-β-Phenylpropionyl-N-methyl-L-tyrosine had no effect at a high concentration.

4 Effects of N-β-phenylpropionyl-L-tyrosine amide (c.c., 3×10^{-7}–10^{-6} M) and N-β-phenylpropionyl-L-tryptophan amide (c.c., 10^{-6} M) were very similar to those of N-β-phenylpropionyl-L-tyrosine and N-β-phenylpropionyl-L-tryptophan respectively.

5 N-β-Phenylpropionyl-*p*-amino-L-phenylalanine (c.c., 3×10^{-5}–10^{-4} M) and N-β-phenylpropionyl-*p*-chloro-L-phenylalanine (c.c., 10^{-4} M) had only a weak effect.

6 It is proposed that the structural features producing the effect are as follows: the active compound has a phenyl or a cyclohexyl group (hydrophobic binding group), after a suitable distance a peptide bond (proton donor and proton acceptor), adjacently a carbonyl group (proton acceptor), and a phenolic hydroxyl or an indolyl imino group (proton donor) in the molecule.

Introduction

It has been reported in previous studies (Takeuchi, Tamura & Sakai, 1979; Takeuchi, Sakai & Tamura, 1979) that two dipeptides composed of aromatic amino acids, L-phenylalanyl-L-tyrosine and L-phenylalanyl-L-tryptophan, could inhibit the spontaneous firing of a giant neurone, TAN (tonically autoactive neurone), identified in the suboesophageal ganglia of *Achatina fulica* Férussac. It has since been shown (Takeuchi & Tamura, 1980; 1981) that two derivatives of the dipeptides mentioned, N-β-phenylpropionyl-L-tyrosine (L-phenylalanyl-L-tyrosine without an amino group) and N-β-phenylpropionyl-L-tryptophan (L-phenylalanyl-L-tryptophan without an amino group), have an inhibitory effect much stronger than that of the dipeptides. From these results, it has been assumed that the following three binding sites of the two compounds are necessary to produce the inhibitory effect: a hydrophobic binding site (phenyl group) in the phenylalanyl part, an anionic binding site (carboxyl group) and a hydrogen binding site (*p*-hydroxyphenyl or indole group) in the tyrosyl or the tryptophyl part.

In the present study, we attempted to elucidate the structural features of these compounds necessary to produce the effect on TAN, by testing effects of their derivatives originally synthesized in our laboratories on the same neurone.

As shown in Figure 1, the derivatives of N-β-phenylpropionyl-L-tyrosine and N-β-phenylpropionyl-L-tryptophan to be examined were synthesized with modifications of the hydrophobic group (I), the chain length of the hydrophobic group (II), the peptide bond (III), the carboxyl group (IV), the chain length of the hydrogen binding group (V) and the hydrogen binding group itself (VI).

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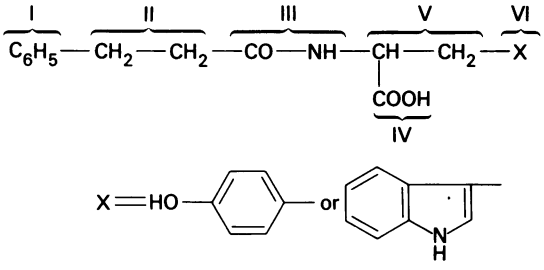

Figure 1 Chemical structure of N-β-phenylpropionyl-L-tyrosine and N-β-phenylpropionyl-L-tryptophan, and portions of this compound, with modifications of which the derivatives were synthesized to elucidate the structure-activity relationships of these compounds on the excitability of TAN (tonically autoactive neurone): (I) hydrophobic binding group; (II) chain length of hydrophobic group; (III) peptide bond; (IV) carboxyl group; (V) chain length of hydrogen binding group; (VI) hydrogen binding group.

Methods

African giant snails, *Achatina fulica* Férussac, were flown in from Okinawa (supplied by Koyo Yakuhin Co. Ltd., Urasoe City). A giant neurone, TAN (tonically autoactive neurone) (Takeuchi, Yokoi, Mori & Kohsaka, 1975; Yokoi, 1980), identified in the suboesophageal ganglia of the animal was used throughout the present experiments. The characteristics of the neurone examined and the electrophysiological methods employed have been described in detail in previous studies (Takeuchi, Yokoi, Mori & Ohmori, 1976; Takeuchi, Yokoi & Hiramatsu, 1977).

The compounds examined in the present study are listed in Table 1. Beside these, the following compounds were also examined: N-carbobenzoxy-O-benzyl-L-tyrosyl-taurine, L-tyrosyl-taurine, N-acetyl-*p*-hydroxy-L-phenylglycine, N-acetyl-*p*-hydroxy-DL-phenylglycine, N-acetyl-*p*-hydroxy-D-phenylglycine, N-benzyl-L-tyrosine, N-benzyl-N-methyl-L-tyrosine, N-methyl-L-tyrosine, N-*t*-butyloxycarbonyl-N-methyl-L-tyrosine dicyclohexylamine salt, N-phenylacetyl-*p*-hydroxy-L-phenylglycine, N-phenylacetyl-DL-veratrylglycine, N-α-naphthylacetyl-DL-veratrylglycine, N-β-naphthalenesulphonyl-DL-veratrylglycine, N-2-fluorenesulphonyl-DL-veratrylglycine, N-phenoxyacetyl-DL-veratrylglycine, N-β-phenylpropionyl-γ-aminobutyric acid, *p*-hydroxy-L-phenylglycine and N-acetyl-L-phenylalanyl-L-tyrosine.

All the compounds examined were analytically pure, and homogeneous on thin-layer chromatography. Optical rotations were determined with a JASCO DIP-140 polarimeter.

Table 1 Structures and effects of N-β-phenylpropionyl-L-tyrosine and their derivatives on the excitability of a molluscan giant neurone, TAN (tonically autoactive neurone), (bath application)

No.	Compound	Chemical structure		Effects on TAN (Conc. M)
	I *Original compounds*			
1	N-β-phenylpropionyl-L-Tyr*	$C_6H_5.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_6H_4.OH$	(I)	3×10^{-7}–10^{-6}
2	N-β-phenylpropionyl-L-Trp*	$C_6H_5.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_8H_6N$	(I)	10^{-6}
3	N-β-phenylpropionyl-L-Phe*	$C_6H_5.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_6H_5$	(I)	10^{-4}
	II *Compounds with a group other than the phenyl group (hydrophobic binding group)*			
4	N-β-cyclohexylpropionyl-L-Tyr	$C_6H_{11}.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_6H_5.OH$	(I)	3×10^{-8}–10^{-7}
5	N-β-cyclohexylpropionyl-L-Trp	$C_6H_{11}.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_8H_6N$	(I)	10^{-6}
6	N-β-cyclohexylpropionyl-L-Phe	$C_6H_{11}.CH_2.CH_2.CO.NH.CH(COOH).CH_2C_6H_5$	(I)	10^{-4}
7	L-Phe-L-Tyr**	$C_6H_5.CH_2.CH(NH_2).CO.NH(COOH).CH_2.C_6H_4.OH$	(I)	1–3×10^{-5}
8	D-Phe-L-Tyr	$C_6H_5.CH_2.CH(NH_2).CO.NH(COOH).CH_2.C_6H_4.OH$	(I)	3×10^{-5}–10^{-4}
9	N-caproyl-L-Tyr	$CH_3.CH_2.CH_2.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_6H_4.OH$	(I)	1–3×10^{-5}
10	N-lauroyl-L-Tyr	$CH_3.(CH_2)_{10}.CO.NH.CH(COOH).CH_2.C_6H_4.OH$	(–)	5.5×10^{-4}
11	N-β-*p*-methylphenylpropionyl-L-Trp	$CH_3.C_6H_4.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_8H_6N$	(I*)	5.7×10^{-4}
12	N-β-*p*-methylphenylpropionyl-L-Tyr	$CH_3.C_6H_4.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_6H_4.OH$	(–)	6.1×10^{-4}
13	N-β-*p*-methylphenylpropionyl-L-Phe	$CH_3.C_6H_4.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_6H_5$	(–)	6.4×10^{-4}
14	N-β-*p*-hydroxyphenylpropionyl-L-Tyr	$OH.C_6H_4.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_6H_4.OH$	(–)	6.1×10^{-4}

	III *Compounds with a different chain length of the hydrophobic group*			
15	N-cinnamoyl-L-Tyr	$C_6H_5.CH{:}CH.CO.NH.CH(COOH).CH_2.C_6H_4.OH$	(I)	3×10^{-5}
16	N-γ-phenylbutyroyl-L-Trp	$C_6H_5.CH_2.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_8H_6N$	(I)	10^{-4}
17	N-γ-phenylbutyroyl-L-Tyr	$C_6H_5.CH_2.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_6H_4.OH$	(−)	6.1×10^{-4}
18	N-γ-phenylbutyroyl-L-Phe	$C_6H_5.CH_2.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_6H_5$	(−)	6.4×10^{-4}
19	N-phenylacetyl-L-Tyr	$C_6H_5.CH_2.CO.NH.CH(COOH).CH_2.C_6H_4.OH$	(I)	10^{-4}
20	N-phenoxyacetyl-L-Phe	$C_6H_5.O.CH_2.CO.NH.CH(COOH).CH_2.C_6H_5$	(−)	6.7×10^{-4}
	IV *Compound with a methylated imino group in the peptide bond*			
21	N-β-phenylpropionyl-N-methyl-L-Tyr	$C_6H_5.CH_2.CH_2.CO.N(CH_3).CH(COOH).CH_2.C_6H_4.OH$	(−)	6.1×10^{-4}
	V *Compounds with a modified carboxyl group (amide and ester)*			
22	N-β-phenylpropionyl-L-Tyr.amide	$C_6H_5.CH_2.CH_2.CO.NH.CH(CONH_2).CH_2.C_6H_4.OH$	(I)	3×10^{-7}–10^{-6}
23	N-β-phenylpropionyl-L-Trp.amide	$C_6H_5.CH_2.CH_2.CO.NH.CH(CONH_2).CH_2.C_8H_6N$	(I)	10^{-6}
24	N-β-phenylpropionyl-L-Tyr.methyl ester	$C_6H_5.CH_2.CH_2.CO.NH.CH(COOCH_3).CH_2.C_6H_4.OH$	(I)	1–3×10^{-6}
25	N-β-pnehylpropionyl-L-Tyr.methylamide	$C_6H_5.CH_2.CH_2.CO.NH.CH(CONHCH_3).CH_2.C_6H_4.OH$	(I)	3×10^{-6}–10^{-5}
	VI *Compounds with a different chain length of the hydrogen binding group*			
26	N-β-phenylpropionyl-L-Hpg	$C_6H_5.CH_2.CH_2.CO.NH.CH(COOH).C_6H_4.OH$	(−)	6.7×10^{-4}
27	N-β-phenylpropionyl-DL-Hpg	$C_6H_5.CH_2.CH_2.CO.NH.CH(COOH).C_6H_4.OH$	(−)	6.7×10^{-4}
28	N-β-phenylpropionyl-D-Hpg	$C_6H_5.CH_2.CH_2.CO.NH.CH(COOH).C_6H_4.OH$	(−)	6.7×10^{-4}
	VII *Compounds with a group other than the* p*-hydroxyphenyl or the indole group (hydrogen binding group)*			
29	N-β-phenylpropionyl-3,4-dihydroxy-L-Phe	$C_6H_5.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_6H_3.(OH)_2$	(I)	3×10^{-6}
30	N-β-phenylpropionyl-*m*-nitro-L-Tyr	$C_6H_5.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_6H_3.(NO_2).OH$	(I)	3×10^{-5}
31	N-β-phenylpropionyl-*p*-amino-L-Phe	$C_6H_5.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_6H_4.NH_2$	(I)	3×10^{-5}–10^{-4}
32	N-β-phenylpropionyl-*p*-chloro-L-Phe	$C_6H_5.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_6H_4Cl$	(I)	10^{-4}
33	N-β-phenylpropionyl-*p*-nitro-L-Phe	$C_6H_5.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_6H_4.NO_2$	(I*)	5.8×10^{-4}
34	N-β-phenylpropionyl-L-His.3/2HCl	$C_6H_5.CH_2.CH_2.CO.NH.CH(COOH).CH_2.C_3H_3N_2$	(−)	5.8×10^{-4}

(I), inhibitory effects. (I*), inhibitory effects, sometimes very slight. (−), no effect. In parentheses (Conc. M), critical or used concentration in M. *, **, effects of the three compounds have been previously reported (*Takeuchi & Tamura, 1980; 1981; **Takeuchi, Tamura & Sakai, 1979). Abbreviations of amino acids follow the recommendations of the IUPAC-IUB Commission on Biochemical nomenclature. Other abbreviation used: Hpg, *p*-hydroxyphenylglycine.

In a typical procedure (N-β-cyclohexylpropionyl-L-tyrosine), to a stirred solution of L-tyrosine methyl ester hydrochloride (7.4 g, 0.03 mol) and triethylamine (3.5 g, 0.035 mol) in chloroform (100 ml) was added 5 g (0.02 mol) of β-cyclohexylpropionic acid N-hydroxysuccinimide ester (m.p. 112°C) which was prepared by condensation of β-cyclohexylpropionic acid and N-hydroxysuccinimide with N,N'-dicyclohexylcarbodiimide. After stirring for 4 h at room temperature, water (100 ml) was added to the reaction mixture. The mixture was washed with 1 N HCl, 5% aqueous sodium bicarbonate solution, and water, and then concentrated *in vacuo*. The syrupy residue thus obtained was dissolved in ethanol (50 ml) and 1 N NaOH (30 ml) was added. The mixture was stirred at room temperature for 4 h, and then adjusted to pH 2.0 with diluted HCl and concentrated *in vacuo* to a small volume, and extracted with ethyl acetate (50 ml × 3). After drying on anhydrous sodium sulphate, ethanol (200 ml) was added to the organic extract to give crystals; yield 4 g (41.8%); m.p. 122–123°C; $[\alpha]_D^{22} + 20.87°$ (c,l, acetone); Found: C, 67.12; H, 8.13; N, 4.34. Calculated for $C_{18}H_{25}O_4N$: C, 67.66; H, 7.91; N, 4.22%.

Most compounds to be examined were dissolved in snail physiological solution (pH 7.5) (Takeuchi, Morimasa, Kohsaka, Kobayashi & Morii, 1973). A few compounds were soluble only in acidic solution. These were first dissolved in a lactic acid solution, and the solution was neutralized by the addition of the physiological solution. Changes in pH were completely avoidable in the present experiments.

The solutions were applied in two ways, by bath application and microdrop application. In the former, an amount of a test solution was applied simply to the ganglia as the screening test. The latter, which was a local application, was performed as follows: the physiological solution covering the ganglia was removed beforehand; a microdrop (about 150 μm in diameter) of a test solution in air at the tip of a second micropipette containing the test solution, was formed under a binocular microscope; then the microdrop was placed carefully on the surface of the neurone examined. This was done only for compounds that had been shown to have an effect by bath application.

The microdrop application of a compound was made in the following three states: in the physiological state; after the ganglia were perfused with the Ca^{2+}-free and Mg^{2+}-rich (24 mM) solution for more than 30 min (Ca^{2+}-free, Mg^{2+}-rich state) in order to minimize synaptic influences; and after perfusion with Cl^--free (replaced with acetate$^-$) solution (Cl^--free state) to detect whether or not the inhibition caused by the compound is due to the permeability increase of the neuromembrane examined to chlorine ions.

The temperature of the experimental room was always kept at 22 ± 1°C.

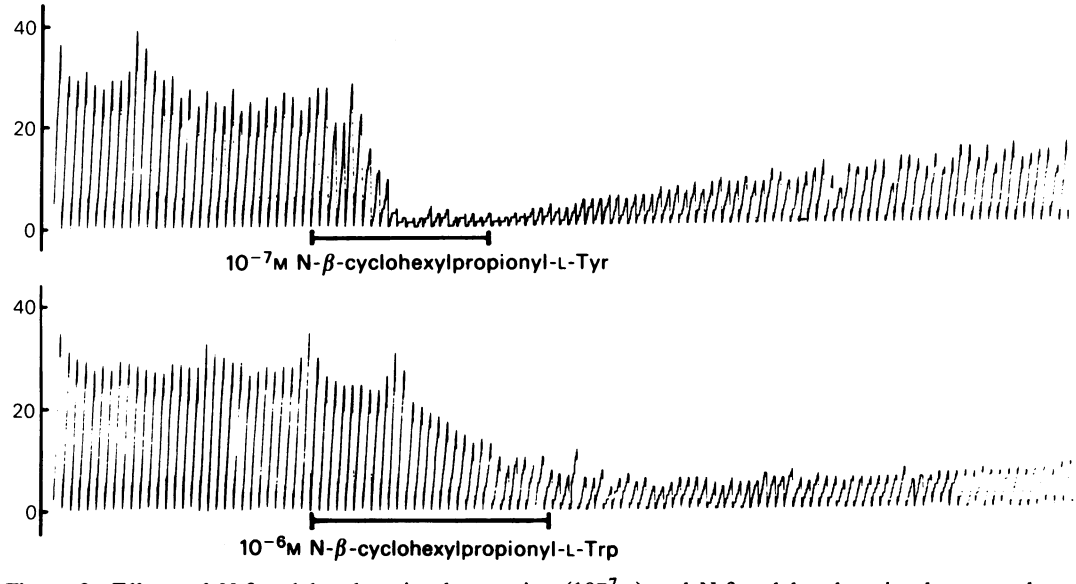

Figure 2 Effects of N-β-cyclohexylpropionyl-L-tyrosine (10^{-7} M) and N-β-cyclohexylpropionyl-L-tryptophan (10^{-6} M) on TAN (bath application). The two recordings were from two TANs. Ordinate scale: number of spike discharges per min. Abscissa scale: time course, each histogram is 1 min.

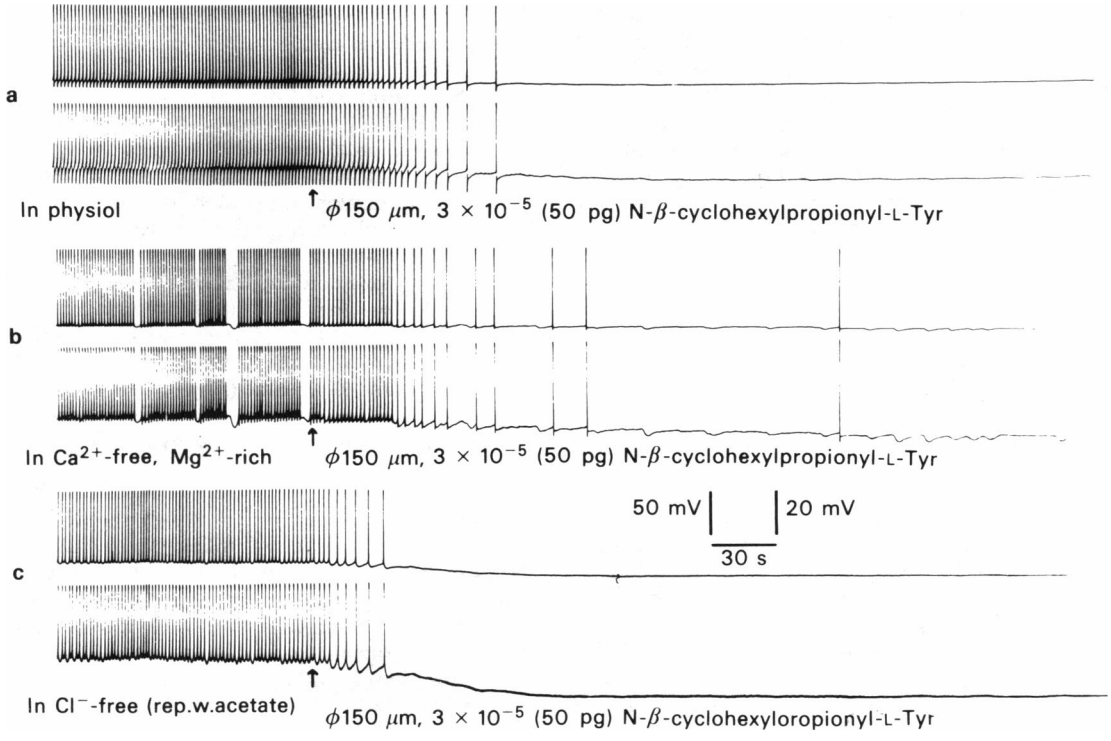

Figure 3 Effects of local (microdrop) application of N-β-cyclohexylpropionyl-L-tyrosine on TAN in the three states. The recordings (a, b and c) were made in three different TANs. Each upper trace of (a), (b) and (c) are the full spike recordings. Each lower trace of (a), (b) and (c) indicate the high gain recordings of each upper trace, where the spike heights were cut electronically. A microdrop (150 μm in diameter) of N-β-cyclohexylpropionyl-L-tyrosine solution at 3×10^{-5} kg/l (containing 50 pg pf the compound) was carefully placed on the TAN surface (arrow): (a) in the physiological state; (b) Ca^{2+}-free, Mg^{2+}-rich (24 mM) state; (c) Cl^{-}-free (replaced with acetate) state. Left vertical bar, calibration for each upper trace (50 mV). Right vertical bar, calibration for each lower trace (20 mV). Horizontal bar, time course (30 s).

Results

The effects of compounds examined by bath application in the present study are summarized in Table 1, in comparison with those of the three N-β-phenylpropionyl compounds previously described (Takeuchi & Tamura, 1980, 1981). In addition, more compounds, as described in Methods were also tested. However, they did not show any effect on the neurone examined.

I Effects of compounds with a group other than the phenyl group (hydrophobic group) of N-β-phenylpropionyl-L-tyrosine

Of the compounds examined in the present study, N-β-cyclohexylpropionyl-L-tyrosine, which has a cyclohexyl group instead of the phenyl group of N-β-phenylpropionyl-L-tyrosine, showed the strongest inhibitory effect on TAN. Its critical concentration (c.c.) was 3×10^{-8}–10^{-7} M, which was much lower than that of N-β-phenylpropionyl-L-tyrosine (c.c., 3×10^{-7}–10^{-6} M). N-β-cyclohexylpropionyl-L-tryptophan also had a marked inhibitory effect (c.c., 10^{-6} M), the intensity of which was almost equal to that of N-β-phenylpropionyl-L-tryptophan (c.c., 10^{-6} M). N-β-Cyclohexylpropionyl-L-phenylalanine as well as N-β-phenylpropionyl-L-phenylalanine had a weak effect (c.c., 10^{-4} M). On the other hand, N-β-*p*-methylphenylpropionyl-L-tyrosine and N-β-*p*-hydroxyphenylpropionyl-L-tyrosine had almost no effect even at high concentrations.

Figure 2 shows the inhibitory effects on TAN of N-β-cyclohexylpropionyl-L-tyrosine at 10^{-7} M and N-β-cyclohexylpropionyl-L-tryptophan at 10^{-6} M. The TAN activity recovered from the inhibition after washing the ganglia with physiological solution. However, recovery took much longer than in the case of a putative neurotransmitter, for example, 5-hydroxytryptamine or dopamine.

Figure 3 indicates the TAN inhibition caused by the application of a microdrop (150 μm in diameter) of N-β-cyclohexylpropionyl-L-tyrosine solution (containing 50 pg of the compound) in the following three conditions: the physiological state, the Ca^{2}-free, Mg^{2}-rich (24 mM) state, and the Cl^{-}-free (replaced with acetate) state. In all three conditions, a microdrop of the compound caused a similar marked hyperpolarization of the neurone.

II Effects of compounds with a hydrophobic group of different chain length

As seen from Table 1, N-cinnamoyl-L-tyrosine, which has a double bond in the chain, had an inhibitory effect (c.c., 3×10^{-5} M) weaker than that of N-β-phenylpropionyl-L-tyrosine.

N-β-phenylbutyroyl-L-tryptophan, which has a longer chain than N-β-phenylpropionyl-L-tryptophan, had a weak effect (c.c., 10^{-4} M). On the other hand, the two compounds analogous to this, n-β-phenylbutyroyl-L-tyrosine and N-β-phenylbutyroyl-L-phenylalanine, had no effect at high concentrations.

N-phenylacetyl-L-tyrosine, with a shorter chain, showed a weak effect (c.c., 10^{-4} M), whereas the analogous N-phenylacetyl-L-phenylalanine had no effect.

III Effects of compound with a methylated imino group in the peptide bond

N-β-phenylpropionyl-N-methyl-L-tyrosine, in which the imino group in the peptide bond is methylated, even at a high concentration lost its effect on TAN (Figure 4). This suggests that the portion of the peptide bond in the structure performs some role to produce the effect.

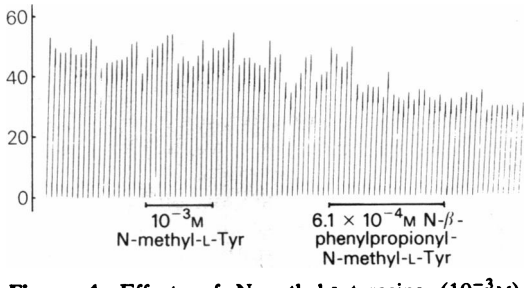

Figure 4 Effects of N-methyl-L-tyrosine (10^{-3} M) and N-β-phenylpropionyl-N-methyl-L-tyrosine (6.1×10^{-4} M) on TAN (bath application). The recording was from one TAN. The recording methods were the same as in Figure 2.

IV Effects of compounds with a modified carboxyl group (amide and ester)

N-β-phenylpropionyl-L-tyrosine amide (c.c., 3×10^{-7}–10^{-6} M) and N-β-phenylpropionyl-L-tryptophan amide (c.c., 10^{-6} M) had marked inhibitory effects with intensities similar to those of N-β-phenylpropionyl-L-tyrosine and N-β-phenylpropionyl-L-tryptophan, respectively (Figure 5).

N-β-phenylpropionyl-L-tyrosine methyl ester (1–3×10^{-6} M) and N-β-phenylpropionyl-tyrosine methylamide (3×10^{-6}–10^{-5} M) had similar effects, but slightly weaker than that of N-β-phenylpropionyl-L-tyrosine (Figure 6).

V Effects of compounds with the hydrogen binding group of a different chain length

N-β-phenylpropionyl-*p*-hydroxy-L-phenylglycine, with a shorter hydrogen binding group chain than N-β-phenylpropionyl-L-tyrosine, had no effect on TAN at a high concentration. The two optical isomers of the compound were also without effect. Some compounds, having a longer chain, for example, N-β-phenylpropionyl-L-homotyrosine, have not yet been synthesized.

VI Effects of compounds with a group other than the p-hydroxyphenyl or the indole group (hydrogen binding group)

N-β-phenylpropionyl-3,4-dihydroxy-L-phenylalanine (L-phenylalanyl-L-DOPA without an amino group), which has one more hydroxyl group in the *p*-hydroxyphenyl group of N-β-phenylpropionyl-L-tyrosine, showed an inhibitory effect (c.c., 3×10^{-6} M), weaker than that of N-β-phenylpropionyl-L-tyrosine. N-β-phenylpropionyl-*m*-nitro-L-tyrosine (c.c., 3×10^{-5} M), with a nitro group in the same group, N-β-phenylpropionyl-*p*-amino-L-phenylalanine (c.c., 3×10^{-5}–10^{-4} M), having an amino group instead of the *p*-hydroxyl group in the group, and N-β-phenylpropionyl-*p*-chloro-L-phenylalanine (c.c., 10^{-4} M), with a chlorine instead of the *p*-hydroxyl group also in the group, showed a much weaker effect. N-β-phenylpropionyl-*p*-nitro-L-phenylalanine, with a nitro group instead of the *p*-hydroxyl group, and N-β-phenylpropionyl-L-histidine, having an imidazole group, had almost no effect even at high concentrations.

Discussion

In the present experiments, the inhibitory effects of N-β-phenylpropionyl-L-tyrosine, N-β-phenylpro-

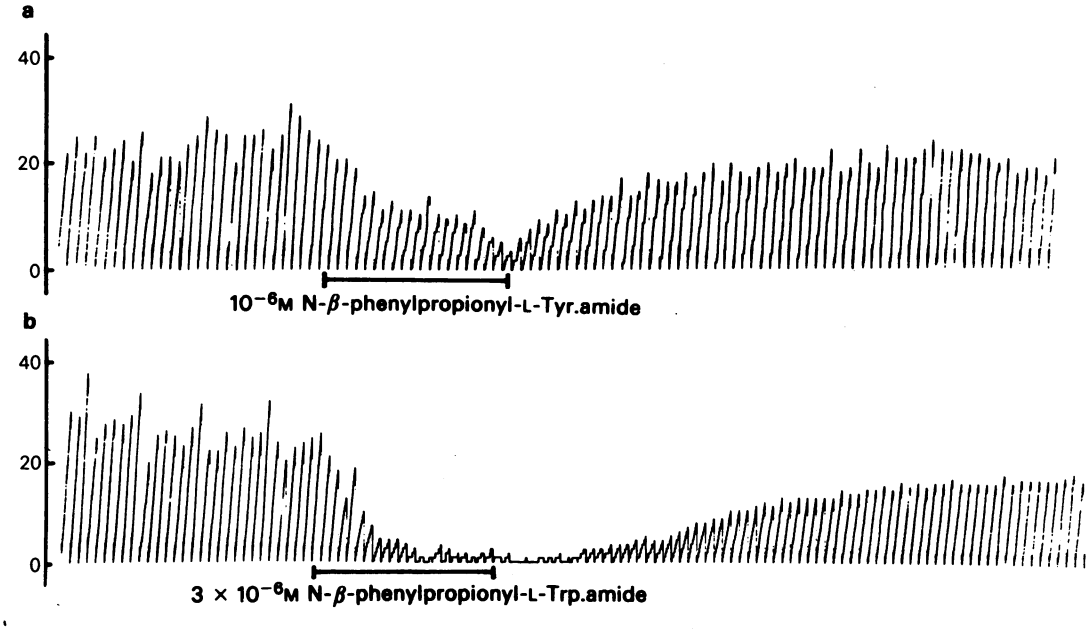

Figure 5 Effects of N-β-phenylpropionyl-L-tyrosine amide (10^{-6} M) and N-β-phenylpropionyl-L-tryptophan amide (3×10^{-6} M) on TAN (bath application). The two recordings were from two TANs. The recording methods were the same as in Figure 2.

pionyl-L-tryptophan and their derivatives were examined on the excitability of an identifiable giant neurone, TAN, of *Achatina fulica* Férussac, in an attempt to elucidate the structural features necessary to produce their effects.

The local (microdrop) application (Takeuchi *et al.*, 1976) of N-β-cyclohexylpropionyl-L-tyrosine, which had the strongest inhibitory effect among the compounds examined in the present study, produced a similar marked TAN hyperpolarization in the physiological state, the Ca^{2+}-free, Mg^{2+}-rich state and the Cl^--free (replaced with acetate) state. This suggests that the TAN inhibition was caused by a direct effect of this compound on the neuromem-

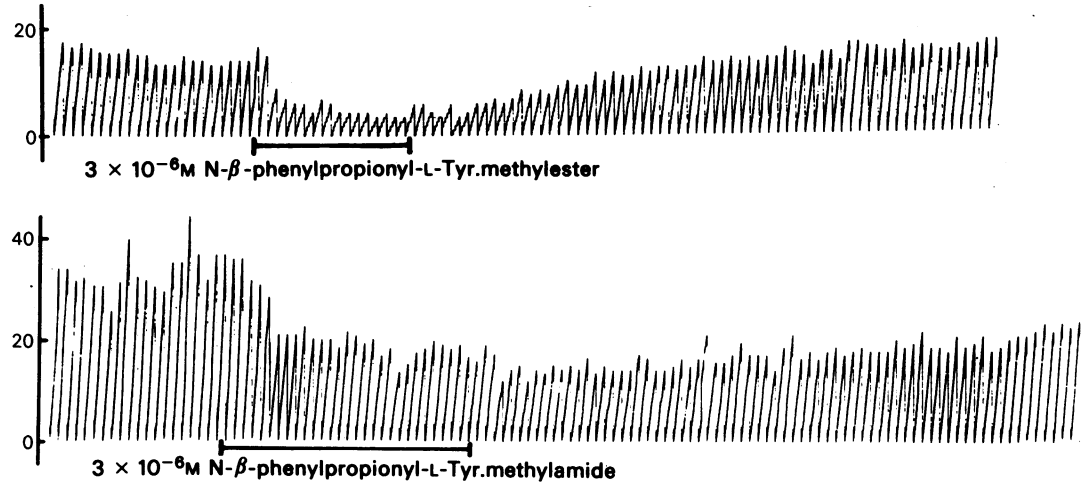

Figure 6 Effects of N-β-phenylpropionyl-L-tyrosine methyl ester (3×10^{-6} M) and N-β-phenylpropionyl-tyrosine methylamide (3×10^{-6} M) (bath application). The recordings were from two TANS. The recording methods were the same as in Figure 2.

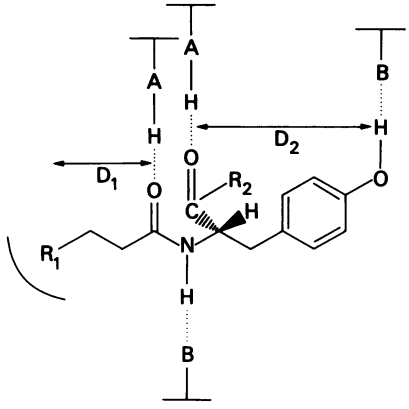

Figure 7 Schematic drawing of the binding sites of N-acyl tyrosine derivatives designed for the specific inhibition of TAN. $R_1 = C_6H_5-$, c-$C_6H_{11}-$; $R_2 = -OH$, $-NH_2$, $-N\text{-}CH_3$, $-OCH_3$.

brane, not by synaptic influences originating from other neurones and that the inhibition was not due to the permeability increase of the neuromembrane to chloride ions (not Cl^--dependent)).

The structural features which would be needed to produce the inhibitory effect on TAN, represented by N-β-cyclohexylpropionyl-L-tyrosine and N-β-phenylpropionyl-L-tyrosine, are drawn schematically in Figure 7. The active compound has a hydrophobic (lipophilic) binding group (R1), such as the phenyl or the cyclohexyl group, at the top. At a suitable distance (D1 in Figure 7, $-CH_2-CH_2-$) from the group, the active compound has a peptide bond, which would interact with the receptor through the hydrogen bonding. In the adjacent position, the active compound has a carbonyl group, which would serve as a proton acceptor. At another suitable distance (D2, $\geq CH-CH_2-C_6H_4-$), the active compound has a phenolic hydroxyl or an indolyl imino group, which would serve as a proton donor, in the end.

In order for the compound to be active, the chiral centre must have the L-configuration (Takeuchi & Tamura, 1980; 1981). The requirement was reconfirmed by the examination of the D-isomer of N-β-phenylpropionyl-L-tryptophan. N-β-phenylpropionyl-D-tryptophan had no effect even at a high concentration.

When a compound had a *p*-hydroxyphenyl or a *p*-methylphenyl group as a hydrophobic group, it completely lost its inhibitory effect. Perhaps the former group decreased the hydrophobicity in this position, and the latter group prevented the binding of the compound to the receptor because of its occupying the receptor site.

Besides the three binding sites, assumed before undertaking the present study to be necessary to produce the described effect, the peptide bond performs a role in which the imino group is perhaps a proton donor, since N-β-phenylpropionyl-N-methyl-L-tyrosine had no effect. Although there is no evidence that the carbonyl group in the peptide bond is necessary to produce the effect, it is supposed that the carbonyl group also would do so as a proton acceptor, simultaneously with the imino group as a proton donor.

It was demonstrated previously (Takeuchi & Tamura, 1980; 1981) that the carboxyl group of N-β-phenylpropionyl-L-tyrosine is necessary to produce the inhibitory effect on TAN, since N-β-phenylpropionyl-tyramine (N-β-phenylpropionyl-L-tyrosine without a carboxyl group) had no effect. It was demonstrated in the present study that both N-β-phenylpropionyl-L-tyrosine amide and N-β-phenylpropionyl-L-trypytophan amide had effects similar to those of their original compounds. With these, it is concluded that the free anionic charge in this group is not necessary. However, the carbonyl group in tyrosine or tryptophan would act as a proton acceptor to produce the effect. The ester and the methylamide of N-β-phenylpropionyl-L-tyrosine showed slightly weaker effects, suggesting that the ester and the methylamide hindered the binding of the compounds to the receptor because of their occupying the receptor site.

The compound has a hydrogen binding group, such as the phenolic hydroxyl or the indolyl imino group, acting as a proton donor in the end. When the *p*-hydroxyphenyl group was replaced with the *p*-aminophenyl or the *p*-chlorophenyl group, the resulting compound had only a weak inhibitory effect on TAN. And when a compound had a *p*-nitrophenyl or an imidazole group instead of the *p*-hydroxyphenyl group, it lost its effect.

A preliminary account of this work was given to the Satellite Symposium of the 8th International Congress of Pharmacology, *Comparative Neuropharmacology*. Reprint requests to H.T., Gifu University, please.

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