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THE EFFECTS OF AROMATIC AMINO ACID DERIVATIVES ON THE EXCITABILITY OF AN IDENTIFIABLE GIANT NEURONE OF THE AFRICAN GIANT SNAIL (*Achatina fulica* Férussac)

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1 The effects of derivatives of aromatic amino acids on the excitability of an identifiable giant neurone (TAN, tonically autoactive neurone) of the African giant snail (*Achatina fulica* Férussac) were examined.

2 The following substances had marked inhibitory effects on TAN using bath application: N-β-phenylpropionyl-L-Tyr and N-β-phenylpropionyl-L-Trp (critical concentration, 3×10^{-7} M), N-β-phenylpropionyl-L-Phe, N-cinnamoyl-DL-Trp and N-phenoxyacetyl-L-Trp (critical concentration, 10^{-5} to 3×10^{-5} M). However, N-β-phenylpropionyl-D-Tyr and N-β-phenylpropionyl tyramine had no effect.

3 Microdrop (150 μm in diameter) application of a solution of N-β-phenylpropionyl-L-Tyr or N-β-phenylpropionyl-L-Trp containing about 100 pg resulted in marked inhibitory effects on TAN. The effect was observed in Ca^{2+}-free, Mg^{2+}-rich (24 mM) solution. Substitution of Cl^- by acetate did not alter the response. This indicates that the two substances act directly on the TAN membrane and not via synaptic influences, and that the inhibition produced by the two substances is not due to the permeability increase of the TAN membrane to Cl^-.

Introduction

In previous papers (Takeuchi, Matsumoto & Mori, 1977; Takeuchi, Matsumoto & Sakai, 1977), it has been reported that chymotrypsin-treated physalaemin, a hypotensive undecapeptide isolated from amphibian skin (Erspamer, Bertaccini & Cei, 1962; Erspamer, Anastasi, Bertaccini & Cei, 1964; Anastasi, Erspamer & Cei, 1964), had marked inhibitory effects on a spontaneously-firing giant neurone, TAN (tonically autoactive neurone), identified in the suboesophageal ganglia of the African giant snail (*Achatina fulica* Férussac). The tripeptide fragment of physalaemin, L-Lys-L-Phe-L-Tyr, is also inhibitory on TAN (Takeuchi, Morimasa & Matsumoto, 1979). Two related dipeptides, L-Phe-L-Tyr and L-Phe-L-Trp, also have marked inhibitory effects on the same neurone (Takeuchi & Sakai, 1977; Takeuchi & Tamura, 1978; Takeuchi, Tamura & Sakai, 1979).

In the present study, we have examined the effects of a number of aromatic amino acid derivatives related to these two dipeptides on TAN excitability and found N-β-phenylpropionyl-L-Tyr and N-β-phenylpropionyl-L-Trp to be the most inhibitory.

Methods

The membrane potential of the giant neurone TAN of the African giant snail (*Achatina fulica* Férussac) was recorded intracellularly with a glass micropipette. The characteristics of the neurone and the electrophysiological methods employed have been described in detail elsewhere (Takeuchi, Yokoi, Mori & Kohsaka, 1975; Takeuchi, Yokoi, Mori & Horisaka, 1976; Takeuchi, Yokoi & Hiramatsu, 1977; Watanabe & Takeuchi, 1977).

All the substances examined in the present study were donated by the Central Research Laboratories of Ajinomoto Co., Inc. For screening, bath application was used. The substances were dissolved in snail physiological solution (Takeuchi, Morimasa, Kohsaka, Kobayashi & Morii, 1973) and applied to the dissected ganglia. Microdrop application (Takeuchi, Yokoi, Mori & Ohmori, 1976) was adopted to apply the most effective two substances locally to the neurone. Using a binocular microscope, oil pressure was used to form a microdrop (150 μm in diameter) of solution containing the substance under test at the tip of a glass micropipette in air which was then placed carefully on the surface of TAN. Microdrop application of the two effective substances was performed under three different conditions: in physiological solution; in a Ca^{2+}-free, Mg^{2+}-rich (24 mM) state (the physiological solution contained 11 mM $CaCl_2$ and 13 mM $MgCl_2$) and in a Cl^--free (replaced with acetate) state. The Ca^{2+}-free, Mg^{2+}-rich state was adopted to minimize synaptic influences; and the Cl^--free state

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Table 1 The effects of aromatic amino acid derivatives on TAN (tonically autoactive neurone) excitability, compared with those of oligopeptides previously reported (bath application)

No.	*Substance*	*Chemical structure*	*Effects on TAN*	*Critical concentration determined in 10 experiments* (M)
1	N-β-phenylpropionyl-L-Tyr	$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CO \cdot NH \cdot CH(COOH) \cdot CH_2 \cdot C_6H_4 \cdot OH$	(I)	3×10^{-7}
2	N-β-phenylpropionyl-D-Tyr	$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CO$-D-Tyr	(−)	$6{\cdot}4 \times 10^{-4}$
3	N-β-phenylpropionyl-tyramine	$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CO \cdot NH \cdot CH_2 \cdot CH_2 \cdot C_6H_4 \cdot OH$	(−)	$7{\cdot}4 \times 10^{-4}$
4	N-β-phenylpropionyl-L-Trp	$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CO \cdot NH \cdot CH(COOH) \cdot CH_2 \cdot C_8H_6N$	(I)	3×10^{-7}
5	N-β-phenylpropionyl-L-Phe	$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CO \cdot NH \cdot CH(COOH) \cdot CH_2 \cdot C_6H_5$	(I)	$10^{-5} \sim 3 \times 10^{-5}$
6	N-carbobenzoxy-L-Tyr	$C_6H_5 \cdot CH_2 \cdot O \cdot CO$-L-Tyr	(I)	6.3×10^{-4}
7	N-cinnamoyl-DL-Trp	$C_6H_5 \cdot CH{:}CH \cdot CO$-DL-Trp	(I)	$10^{-5} \sim 3 \times 10^{-5}$
8	N-phenoxyacetyl-L-Trp	$C_6H_5 \cdot O \cdot CH_2 \cdot CO$-L-Trp	(I)	$10^{-5} \sim 3 \times 10^{-5}$
9	N-α-naphthylacetyl-DL-Trp	$C_{10}H_7 \cdot CH_2 \cdot CO$-DL-Trp	(I)	5.4×10^{-4}
10	N-α-naphthylacetyl-*m*-nitro-L-Tyr	$C_{10}H_7 \cdot CH_2 \cdot CO$-L-Tyr$\cdot NO_2$	(I*)	5.1×10^{-4}
11	N-phenylacetyl-DL-Trp	$C_6H_5 \cdot CH_2 \cdot CO$-DL-Trp	(I*)	6.2×10^{-4}
12	L-Lys-L-Phe-L-Tyr*		(I)	$1.1 \times 10^{-5} \sim 2.2 \times 10^{-5}$
13	L-Phe-L-Tyr*		(I)	$1.5 \times 10^{-5} \sim 3.0 \times 10^{-5}$
14	L-Phe-L-Trp$\cdot CH_3COOH$**		(I)	$2.4 \times 10^{-5} \sim 7.3 \times 10^{-5}$

* Takeuchi & Sakai, 1977. ** Takeuchi & Tamura, 1978. (I), inhibitory effect. (I*), inhibitory effect, slight in some cases. (−) no effect in 3 experiments at the concentration indicated.

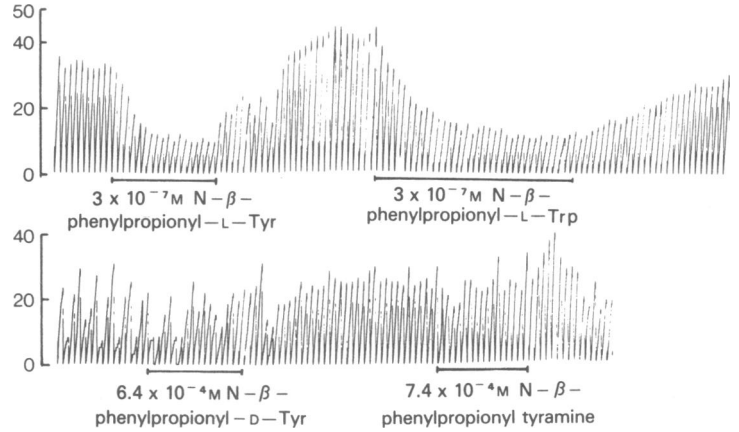

Figure 1 The effects of aromatic amino acid derivatives (bath application) on TAN (tonically autoactive neurone) excitability of the African giant snail. Two traces were recorded from two TANs. Ordinate scale: number of spike discharges per min, abscissa scale: time course, each histogram represents 1 min. N-β-phenylpropionyl-L-Tyr 3×10^{-7} M, N-β-phenylpropionyl-L-Trp 3×10^{-7} M, N-β-phenylpropionyl-D-Tyr 6.4×10^{-4} M and N-β-phenylpropionyl tyramine 7.4×10^{-4} M were applied.

to determine whether the inhibition produced by the substance examined is due to the permeability increase of the TAN membrane to Cl^- (Cl^--dependent).

The pH of the solutions was always 7.5, and the temperature of the experimental room was maintained at 23 ± 1°C. Snails were flown from Okinawa.

Results

The effects of the substances examined in the present study are summarized in Table 1. Of these, the two newly synthesized derivatives of aromatic amino acids, N-β-phenylpropionyl-L-Tyr (L-Phe-L-Tyr without an amino group) and N-β-phenylpropionyl-L-Trp (L-Phe-L-Trp without an amino group), had the strongest inhibitory effects on TAN excitability (critical concentration, 3×10^{-7} M). Their effects were much stronger than those of the corresponding oligopeptides. However, N-β-phenylpropionyl-D-Tyr and N-β-phenylpropionyl-tyramine, had no effect, even at high concentrations (6.4 or 7.4×10^{-4} M). Three other derivatives, N-β-phenylpropionyl-L-Phe, N-cinnamoyl-DL-Trp and N-phenoxyacetyl-L-Trp also had marked inhibitory effects (critical concentration, 10^{-5} to 3×10^{-5} M) which were weaker than those of the two most effective substances. At high concentrations (5.1×10^{-4} to 6.3×10^{-4} M) four other derivatives, N-carbobenzoxy-L-Tyr, N-α-naphthylacetyl-DL-Trp, N-α-naphthylacetyl-*m*-nitro-L-Tyr, N-phenylacetyl-DL-Trp, showed slight inhibitory effects.

The following aromatic amino acid derivatives were also examined in this study: N-acetyl-DL-Tyr, N-acetyl-L-Trp, N-chloroacetyl-DL-Trp, N-chloroacetyl-*m*-nitro-L-Tyr, N-β-chloropropionyl-DL-Trp, N,O-diacetyl-DL-Tyr, N-9-fluorenylacetyl-DL-Trp, N-phenylacetyl-*m*-nitro-Tyr, N-propionyl-L-Trp, N-cinnamoyl-L-Phe, N-furfuryl-L-Phe, N-β-naphthalenesulphonyl-*p*-nitro-L-Phe, N-α-naphthylacetyl-L-Phe and N-*p*-toluenesulphonyl-DL-Trp and shown to have no effect (4.9×10^{-4} to 9.0×10^{-4} M).

Figure 1 shows the inhibitory effects of the two most effective substances, N-β-phenylpropionyl-L-Trp and N-β-phenylpropionyl-L-Tyr, at 3×10^{-7} M on the frequency of TAN spike discharges. At this concentration, the spike frequency decreased gradually, but was not abolished. After washing, the spike frequency slowly returned to the control value. At 10^{-6} M, the inhibitory action on spike discharges was almost complete.

Figure 2 demonstrates the effects of microdrop application of N-phenylpropionyl-L-Tyr on the TAN membrane potential in the control solution, in Ca^{2+}-free, Mg^{2+}-rich (24 mM) media and in Cl^--free media in which this anion was replaced with acetate. In each experiment, the membrane potential began to hyperpolarize within a few seconds of the microdrop application. One minute later, the spontaneous spike discharges ceased. After washing with physiological solution the membrane potential gradually returned to the control level.

Figure 3 shows similar results obtained with N-β-phenylpropionyl-L-Trp. From the results it was concluded that these substances act directly on the TAN membrane and not trans-synaptically, and that inhibi-

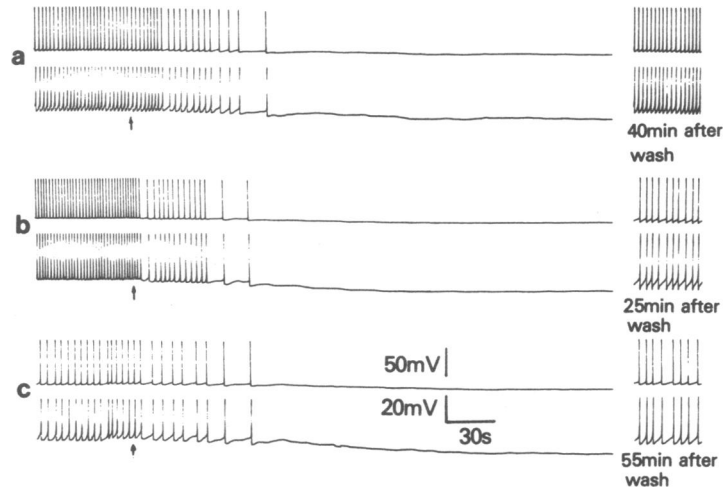

Figure 2 The effects of N-β-phenylpropionyl-L-Tyr (microdrop application) on the TAN membrane potential of African giant snail: (a), (b) and (c) were recorded from a TAN. Upper traces of (a), (b) and (c), full spike recordings of the TAN membrane potential. Lower traces of (a), (b) and (c), high gain recordings of the upper traces (spike peaks were cut by an electronic voltage clipper). In (a), (b) and (c), a microdrop (about 150 μm in diameter) of 2×10^{-4} M N-β-phenylpropionyl-L-Tyr solution (total amount of the applied compound is about 112 pg) was placed on the TAN surface (arrow). (a) Physiological state; (b) Ca^{2+}-free, Mg^{2+}-rich (24 mM) state; (c) Cl^{-}-free state (replaced by acetate). Upper vertical bar, calibration for each upper trace (50 mV). Lower vertical bar, calibration for each lower trace (20 mV). Horizontal bar, time course (30 s.).

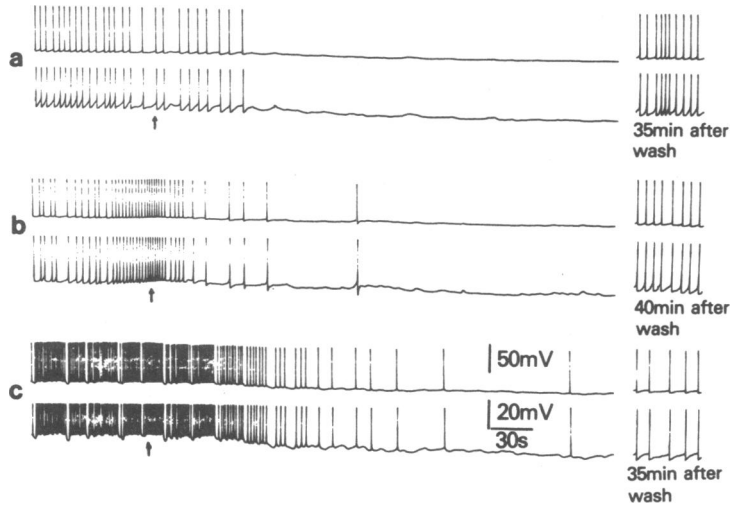

Figure 3 The effects of N-β-phenylpropionyl-L-Trp (microdrop application) on the TAN membrane potential of African giant snail: (a) and (b) were recorded from the same TAN; (c) was recorded from another TAN. Recording methods and calibrations were the same as those of Figure 2. In (a), (b) and (c), a microdrop (about 150 μm in diameter) of 2×10^{-4} M N-β-phenyl-propionyl-L-Trp solution (total amount of the applied compound is calculated to be about 121 pg) was placed on the TAN surface (arrow). (a): Physiological state; (b): Ca^{2+}-free, Mg^{2+}-rich (24 mM) state; (c): Cl^{-}-free state (replaced by acetate).

tion is not due to an increase in membrane permeability to chloride ions.

Discussion

As reported previously (Takeuchi & Sakai, 1977; Takeuchi & Tamura, 1978; Takeuchi *et al* 1979), the three oligopeptides, L-Lys-L-Phe-L-Tyr, L-Phe-L-Tyr and L-Phe-L-Trp, have a marked inhibitory effect on TAN excitability, and L-Tyr-L-Phe, L-Trp-L-Phe, L-Phe, L-Tyr and L-Trp have no effect. Since the two aromatic amino acid derivatives, N-β-phenylpropionyl-L-Tyr and N-β-phenylpropionyl-L-Trp, are more potent inhibitors than the corresponding oligopeptides as demonstrated in the present study, the following three side groups may be necessary for this effect: a phenyl (hydrophobic) group in the phenylpropionyl, a *p*-hydroxyphenyl or indole group and a carboxyl group in the tyrosyl or the tryptophyl. N-β-phenylpropionyl-D-Tyr has no effect, suggesting that the stereoisomerism of these substances may be important. Since N-β-phenylpropionyl tyramine has no effect, the carboxyl group can be regarded as essential. On the other hand, the amino group of the effective oligopeptides is not.

The TAN inhibition produced by N-β-phenylpropionyl-L-Tyr and N-β-phenylpropionyl-L-Trp is unaltered by the replacement of Cl^- in the external media by acetate as is that of L-Phe-L-Tyr, L-Phe-L-Trp and dopamine. However, the inhibition of this neurone caused by γ-aminobutyric acid and acetylcholine appear to be Cl^--dependent (Watanabe & Takeuchi, 1977). Since the critical concentration of dopamine required to inhibit TAN is about 10^{-4} M (Takeuchi, Tamura & Sakai, 1979), almost 300 times higher than that of N-β-phenylpropionyl-L-Tyr and N-β-phenylpropionyl-L-Trp, these two aromatic amino acid derivatives cannot be regarded as dopamine receptor agonists on TAN. Presumably these substances or other substances with similar groups could play some physiological role in the nervous system.

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