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Effects of glutamic acid analogues on identifiable giant neurones, sensitive to β-hydroxy-L-glutamic acid, of an African giant snail (*Achatina fulica* Férussac)

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1 The effects of the seven glutamic acid analogues, α-kainic acid, α-allo-kainic acid, domoic acid, erythro-L-tricholomic acid, DL-ibotenic acid, L-quisqualic acid and allo-γ-hydroxy-L-glutamic acid were examined on six identifiable giant neurones of an African giant snail (*Achatina fulica* Férussac).

2 The neurones studied were: PON (periodically oscillating neurone), d-RPLN (dorsal-right parietal large neurone), VIN (visceral intermittently firing neurone), RAPN (right anterior pallial neurone), FAN (frequently autoactive neurone) and v-RCDN (ventral-right cerebral distinct neurone). Of these, d-RPLN and RAPN were excited by the two isomers (erythro- and threo-) of β-hydroxy-L-glutamic acid (L-BHGA), whereas PON, VIN, FAN and v-RCDN were inhibited. L-Glutamic acid (L-Glu) had virtually no effect on these neurones.

3 α-Kainic acid and domoic acid showed marked excitatory effects, similar to those of L-BHGA, on d-RPLN and RAPN. Their effective potency quotients (EPQs), relative to the more effective isomer of L-BHGA were: 0.3 for both substances on d-RPLN, and 1 for α-kainic acid and 3–1 for domoic acid on RAPN. α-Kainic acid also had excitatory effects on FAN and v-RCDN (EPQ for both: 0.3), which were inhibited by L-BHGA but excited by γ-aminobutyric acid (GABA).

4 Erythro-L-tricholomic acid showed marked effects, similar to those of L-BHGA, on VIN (EPQ: 0.3) and RAPN (EPQ: 3–1), but produced weaker effects on PON and d-RPLN (EPQ: 0.1).

5 DL-Ibotenic acid produced marked effects, similar to those of L-BHGA, on PON, VIN (EPQ for both: 1) and RAPN (EPQ:1–0.3), but had weak effects on d-RPLN (EPQ: <0.1) and FAN (EPQ: 0.1). It had excitatory effects on v-RCDN (EPQ: 0.1). This neurone was inhibited by L-BHGA but excited by GABA.

6 L-Quisqualic acid showed the same effects as L-BHGA on all of the neurones examined (EPQ range 30–0.1). It was the most potent of the compounds tested on RAPN (EPQ: 30–10), FAN (EPQ:30) and v-RCDN (EPQ:3).

7 α-Allo-kainic acid and allo-γ-hydroxy-L-glutamic acid had no obvious effect on any of the neurones examined.

8 As described above, the responses of the neurones examined to these substances varied widely. However, L-quisqualic acid generally had effects on the neurones similar to those of L-BHGA; the L-BHGA-excited neurones were also excited by α-kainic acid and domoic acid.

Introduction

Although more than twenty giant neurones have already been identified in the ganglia of an African giant snail (*Achatina fulica* Férussac), none have been shown to be sensitive to L-glutamic acid (L-Glu). However, several giant neurones are sensitive to β-hydroxy-L-glutamic acid (L-BHGA) (Takeuchi *et al.*, 1976; Takeuchi & Yamaoto, 1982; Ku & Takeuchi, 1983a; b; Ku *et al.*, 1985).

In a previous paper (Watanabe *et al.*, 1985), the effects of the four (erythro-L-, threo-L-, erythro-D- and

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threo-D-) stereoisomers of BHGA on the six identifiable giant neurones of *Achatina fulica* Férussac were compared. The erythro-L- and threo-L-isomers affected these neurones, whereas the two D-isomers were virtually ineffective. Three of the six neurones were more sensitive to erythro-L-BHGA than to threo-L-BHGA (erythro-L-type), whereas two neurones were more sensitive to threo-L-BHGA (threo-L-type). Another neurone was equally sensitive to both erythro-L- and threo-L-BHGA (combined type).

Certain L-Glu analogues have been isolated from their natural sources and their structures identified (Takemoto, 1978). α-Kainic acid and domoic acid were originally isolated from the seaweeds, *Digenea simplex* and *Chondria armata*, respectively; erythro-L-tricholomic acid and DL-ibotenic acid were obtained from the two mushrooms, *Tricholoma muscarium* and *Amanita strobiliformis*, respectively. L-Quisqualic acid was isolated from seeds of the plant, *Quisqualis indica*.

In the present study, a comparison was made of the effects of seven L-Glu analogues, including the naturally-occurring substances mentioned above, on six giant neurones sensitive to L-BHGA, in order to elucidate the pharmacological features of the neuronal chemoreceptors for L-BHGA.

Methods

The African giant snail (*Achatina fulica* Férussac) was flown in from Okinawa (supplied by Koyo Yakuhin Co., Ltd). The ganglia together with some peripheral nerves were dissected from the animal. The following six identifiable giant neurones, which were sensitive to β-hydroxy-L-glutamic acid ((2S)-2-amino-3-hydroxy-pentanedioic acid, L-BHGA), were used in the present study: PON (periodically oscillating neurone), d-RPLN (dorsal-right parietal large neurone) and RAPN (right anterior pallial neurone) in the right parietal ganglion; VIN (visceral intermittently firing neurone) and FAN (frequently autoactive neurone) in the visceral ganglion; and v-RCDN (ventral-right cerebral distinct neurone) in the right cerebral ganglion.

A glass microelectrode was implanted into the soma of these neurones. The membrane potential was registered by a pen-galvanometer, and the number of spike discharges per minute was recorded by a spike counter. The electrophysiological methods used were described in detail in previous studies (Takeuchi *et al.*, 1975; 1976; 1977).

The following substances, analogues of L-glutamic acid ((2S)-2-aminopentanedioic acid, L-Glu), were tested in the present study: α-kainic acid ((2S, 3S, 4S)-2-carboxy-4-isopropenyl-pyrrolidine-3-acetic acid), α-allo-kainic acid ((2S, 3S, 4R)-2-carboxy-4-isopropenyl-pyrrolidine-3-acetic acid), domoic acid ((2S, 3S, 4S)-2-carboxy-4-(1-methyl-5(R)-carboxy-1-(Z), 3(E)-hexadienyl)-pyrrolidine-3-acetic acid), erythro-L-tricholomic acid (erythro-L-α-amino-3-oxo-isoxazolidine-5-acetic acid), DL-ibotenic acid (DL-α-amino-3-oxo-4-isoxazoline-5-acetic acid), L-quisqualic acid (L-α-amino-β-(3, 5-dioxo-1, 2, 4-oxadiazolidine-2-yl)-propionic acid) and allo-α-hydroxy-L-glutamic acid ((2S, 4S)-2-amino-4-hydroxypentanedioic acid, allo-L-GHGA). These substances were obtained from their natural sources or synthesized in our laboratories (Takemoto, 1978; Ofune & Tomita, 1982), and it was confirmed that they were analytically pure. Their chemical structures are shown in Figure 1. Each substance was dissolved in snail physiological solution (Takeuchi *et al.*, 1973), and applied directly to the dissected ganglia by addition to the bathing medium.

To compare the effective potency of each of these substances for each neurone, the minimum effective concentration (MEC) was determined as follows: initially the effects of the substances at the screening concentration of 10^{-3}M were examined; then, if marked effects were obtained, the concentration was serially decreased by 3.3 or 3.0, down to the lowest concentration at which the substance showed appreciable effects in the majority of trials. The effective potency quotient (EPQ) of each substance for each neurone was then calculated by taking the proportion of the MEC value of the more effective (erythro- or threo-) isomer of L-BHGA for the neurone, which was determined in a previous study (Watanabe *et al.*, 1985), to the MEC of the given substance. As the standard, the EPQ of the more effective isomer of L-BHGA was defined as 1. If the MEC value of the given substance was, for example, 10 times higher than that of the more effective isomer of L-BHGA, the EPQ of the substance was expressed as 0.1.

Results

Table 1 summarizes the experimental results obtained in the present study, and shows for comparison, those obtained with L-BHGA in an earlier study (Watanabe *et al.*, 1985). The six identifiable giant neurones examined have been classified into erythro-L-, threo-L- and combined types by their sensitivities to the isomers (erythro- and threo-) of L-BHGA as reported in the previous investigation. The effects of the seven L-Glu analogues on these neurones, their effective potency quotients (EPQs) as compared to the potency of the more effective isomer of L-BHGA for each neurone, and the numbers of trials, are given in Table 1. Data for the substances which showed effects similar to those of L-BHGA, are underlined.

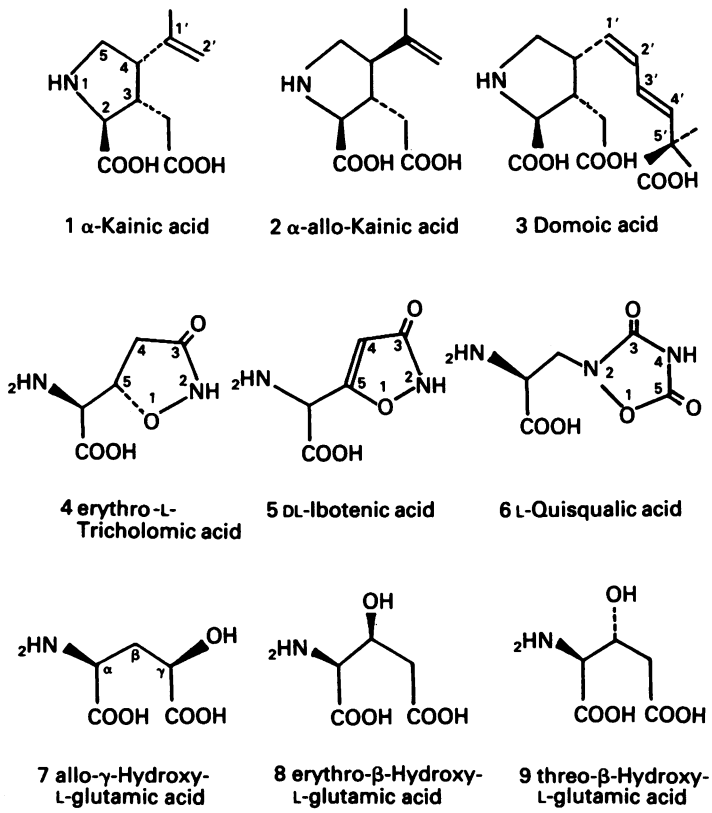

Figure 1 Chemical structures of the glutamic acid analogues examined in the present study, in comparison with two isomers of β-hydroxy-L-glutamic acid (L-BHGA). (1) α-Kainic acid, with the carbon number of the pyrrolidine ring and the 1'-substituent. (2) α-Allo-kainic acid. (3) Domoic acid, with the carbon number of the 1'-substituent. (4) Erythro-L-tricholomic acid, with the carbon number of the isoxazolidine ring. (5) DL-Ibotenic acid, with the carbon number of the isoxazoline ring. (6) L-Quisqualic acid, with the carbon number of the oxadiazolidine ring. (7) Allo-γ-hydroxy-L-glutamic acid, with the carbon number of the structure. (8) Erythro-L-BHGA. (9) Threo-L-BHGA.

Effects on erythro-L-type neurones

The three neurones examined, PON (periodically oscillating neurone), d-RPLN (dorsal-right parietal large neurone) and VIN (visceral intermittently firing neurone), were more sensitive to erythro-L-BHGA than to threo-L-BHGA. Of these neurones, PON and VIN were inhibited by both isomers of L-BHGA, whereas d-RPLN was excited.

Figure 2 shows the marked inhibitory effects of DL-ibotenic acid and L-quisqualic acid on the PON membrane potential. The application of these two substances at 10^{-4}M produced, like L-BHGA, a gradual and marked hyperpolarization of the PON membrane potential. Their minimum effective concentrations (MECs) were almost comparable to that of erythro-L-BHGA (3×10^{-5}M) and were as follows: 3×10^{-5}M for DL-ibotenic acid (EPQ as compared to erythro-L-BHGA: 1), and $1-3 \times 10^{-5}$M for L-quisqualic acid (EPQ: 3–1). Erythro-L-tricholomic acid was less inhibitory on PON (EPQ: 0.1) than the two substances mentioned; α-kainic acid and domoic acid at the high concentration of 10^{-3}M produced slight and unstable results (EPQ:0.03), which could be caused by synaptic effect; α-allo-kainic acid and allo-γ-hydroxy-L-glutamic acid (allo-L-GHGA) at 10^{-3}M had no effect.

Figure 3 represents the effects of the L-Glu analogues examined on the membrane potential of d-RPLN. α-Kainic acid, domoic acid and L-quisqualic acid showed markedly excitatory effects on the neurone, similar to those of L-BHGA, L-Quisqualic

Table 1 Effective potency quotients (EPQs) of glutamic acid analogues, in comparison with those of two stereoisomers of β-hydroxy-L-glutamic acid (L-BHGA), on six identifiable giant neurones of *Achatina fulica* Férussac

	Erythro-L-type			*Combined type*	*Threo-L-type*	
Identifiable neurones	PON	d-RPLN	VIN	RAPN	FAN	v-RCDN
Effects of L-BHGA*	I	E	I	E	I	I
EPQ of L-BHGA (erythro:threo)*	1:0.3–0.1	1:0.1	1:0.3–0.1	1:1	0.3:1	0.1:1
MEC of stronger L-BHGA*	3×10^{-5}M	10^{-4}M	3×10^{-5}M	10^{-4}M	10^{-4}M	10^{-5}M
No. Substance	*Effective potency quotient* (effect, *n*)					
1 α-Kainic acid	0.03 (Us, 9)	0.3 (E,21)	<0.03 (Us/–,12)	1 (E,13)	0.3 (E,25)	0.3 (E,20)
2 α-allo-Kainic acid	(–,6)	(–,5)	<0.03 (I/–,10)	(–,4)	(–,5)	(–,5)
3 Domoic acid	0.03 (Us,9)	0.3 (E,16)	0.1–0.03 (I,22)	3–1 (E,12)	(–,9)	0.1–0.03 (E,15)
4 e-L-Tricholomic acid	0.1 (I,23)	0.1 (E,16)	0.3 (I,18)	3–1 (E,15)	<0.1 (Us/–,9)	0.01 (Bi,8)
5 DL-Ibotenic acid	1 (I,22)	<0.1 (E/–,16)	1 (I,17)	1–0.3 (E,21)	0.1 (I,21)	0.1 (E,16)
6 L-Quisqualic acid	3–1 (I,27)	1 (E,18)	1–0.3 (I,36)	30–10 (E,41)	30 (I,16)	3 (I,15)
7 allo-L-GHGA	(–,4)	(–,3)	(–,7)	0.1 (E,10)	(–,4)	<0.01 (I/–,7)

EPQ, effective potency quotient as compared to the potency of the more effective isomer of L-BHGA for each neurone. MEC, minimum effective concentration. *Watanabe *et al.*, 1985. Beneath each EPQ is shown the effect of the substance tested and the number (*n*) of trials. Abbreviations are as follows: E, excitatory effects. I, inhibitory effects. Us, unstable effects. Bi, biphasic (excitatory followed by inhibitory) effects. E/–, excitatory or no effect at screening concentration (SC) of 10^{-3}M. I/–, inhibitory or no effect at SC. Us/–, unstable or no effect at SC. –, no effect at SC. Data for the substances which showed effects similar to those of L-BHGA, are underlined. e-L-, erythro-L-. L-GHGA, γ-hydroxy-L-glutamic acid. Abbreviated names of the neurones listed here are described in the text.

acid was equipotent with erythro-L-BHGA (MEC: 10^{-4}M, EPQ:1), whereas α-kainic acid and domoic acid were somewhat less potent (both EPQs: 0.3). Erythro-L-tricholomic acid had weak excitatory effects (EPQ: 0.1); DL-ibotenic acid at a concentration of 10^{-3}M produced a slight but sometimes undetectable, excitation of d-RPLN (EPQ: <0.1); α-allo-kainic acid and allo-L-GHGA at 10^{-3}M were ineffective.

Figure 4 shows effects of DL-ibotenic acid, erythro-L-tricholomic acid and L-quisqualic acid on VIN, with respect to the number of its spike discharges. In general, VIN responded like PON to the three substances; the three had marked inhibitory effects, similar to those of L-BHGA, on VIN; their MECs were: 3×10^{-5}M for DL-ibotenic acid (EPQ:1), 10^{-4}M for erythro-L-tricholomic acid (EPQ: 0.3) and 3×10^{-5}–10^{-4}M for L-quisqualic acid (EPQ: 1–0.3). α-Kainic acid and domoic acid at 10^{-3}M had only slight and unstable effects (EPQs: <0.03 and 0.1–0.03 respectively); α-allo-kainic acid and allo-L-GHGA at 10^{-3}M had very little effect.

Effects on combined type neurone

RAPN (right anterior pallial neurone) was equally excited by erythro-L- and threo-L-isomers of BHGA. Figure 5 shows the RAPN membrane potential changes caused by the L-Glu analogues. RAPN was highly excited by L-quisqualic acid, the effects of which were similar to and much more potent than those of L-BHGA; the MEC of L-quisqualic acid was 3×10^{-6}–10^{-5}M (EPQ: 30–10). The neurone was also excited by the other four substances, α-kainic acid (EPQ: 1), domoic acid (EPQ: 3–1), erythro-L-tricholomic acid (EPQ: 3–1) and DL-ibotenic acid (EPQ: 1–0.3). α-Allo-kainic acid at 10^{-3}M had no effect; allo-L-GHGA, at the same concentration, showed slight excitatory effects (EPQ: 0.1).

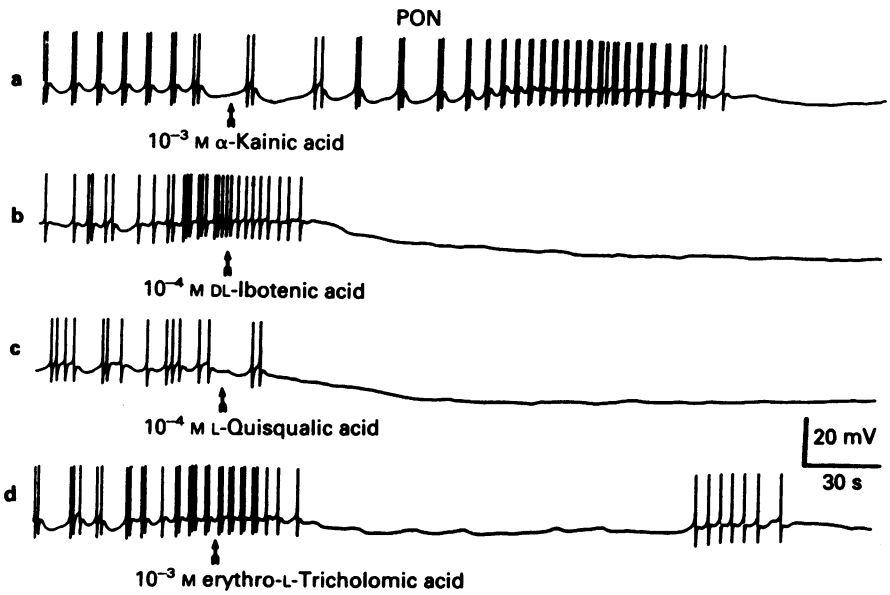

Figure 2 Effects of α-kainic acid at 10^{-3}M (a), DL-ibotenic acid at 10^{-4}M (b), L-quisqualic acid at 10^{-4}M (c) and erythro-L-tricholomic acid at 10^{-3}M (d) on PON (periodically oscillating neurone) membrane potential. The spike heights were cut electronically. Vertical bar, calibration (20 mV). Horizontal bar, time course (30 s).

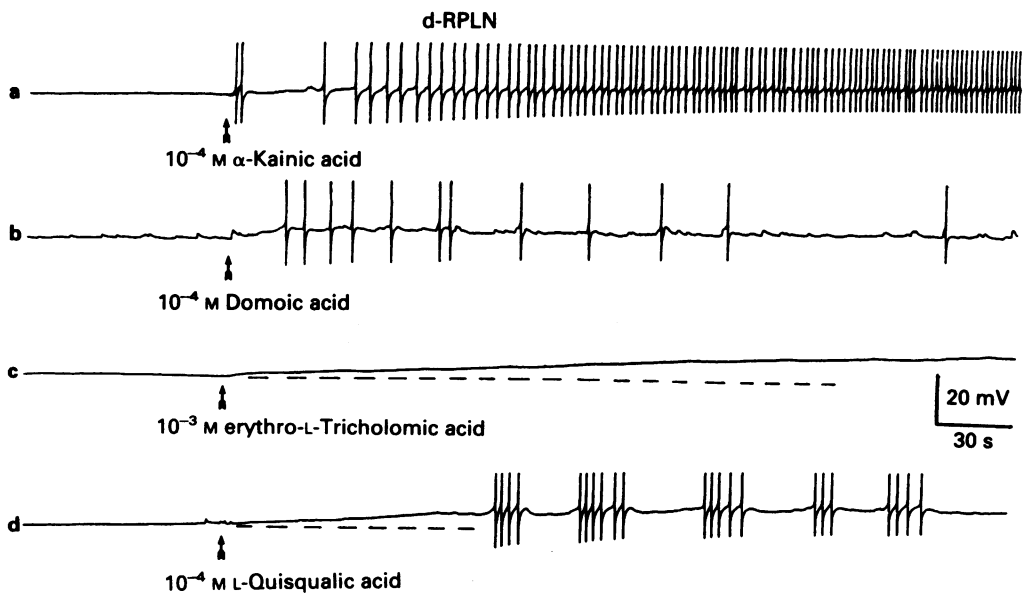

Figure 3 Effects of α-kainic acid at 10^{-4}M (a), domoic acid at 10^{-4}M (b), erythro-L-tricholomic acid at 10^{-3}M (c) and L-quisqualic acid at 10^{-4}M (d) on d-RPLN (dorsal-right parietal large neurone) membrane potential. The spike heights were cut electronically. Vertical bar, calibration (20 mV). Horizontal bar, time course (30 s).

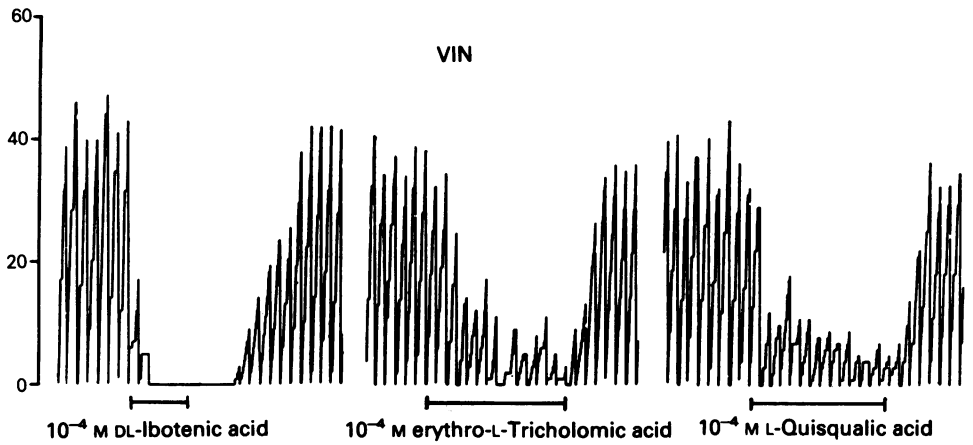

Figure 4 Effects of DL-ibotenic acid at 10^{-4}M, erythro-L-tricholomic acid at 10^{-4}M and L-quisqualic acid at 10^{-4}M on VIN (visceral intermittently firing neurone) excitability. Ordinate scale, number of spike discharges per min. Abscissa scale, time course, each histogram is 1 min.

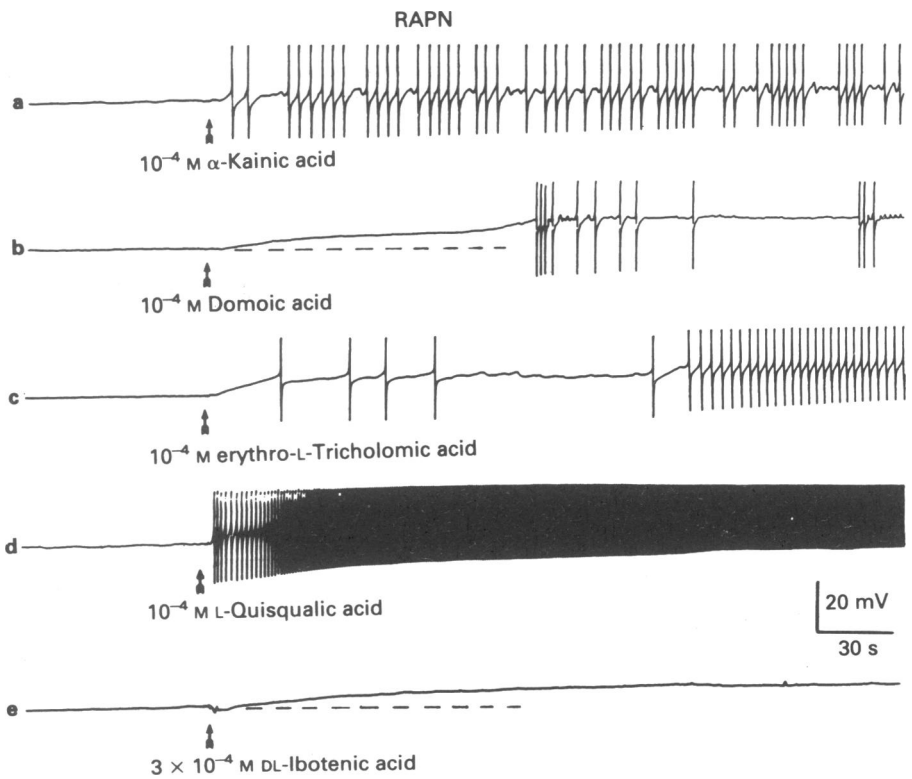

Figure 5 Effects of α-kainic acid at 10^{-4}M (a), domoic acid at 10^{-4}M (b), erythro-L-tricholomic acid at 10^{-4}M (c), L-quisqualic acid at 10^{-4}M, (d) and DL-ibotenic acid at 3×10^{-4}M (e) on RAPN (right anterior pallial neurone) membrane potential. The spike heights were cut electronically. Vertical bar, calibration (20 mV). Horizontal bar, time course (30 s).

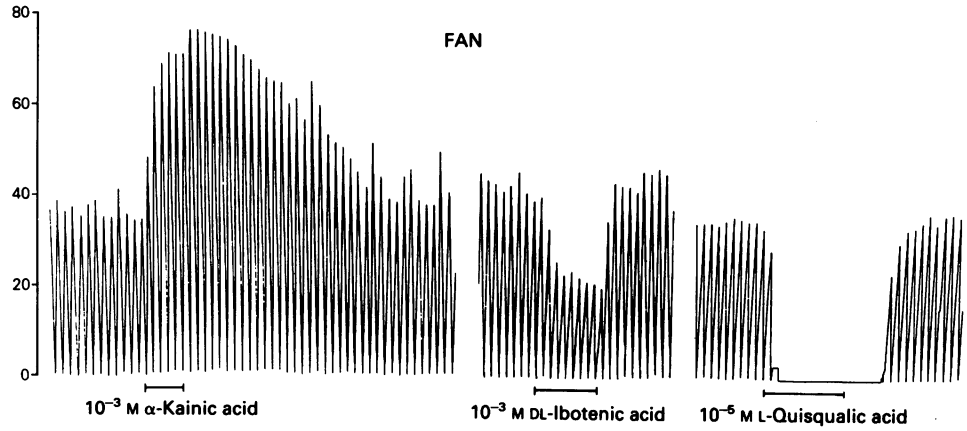

Figure 6 Effects of α-kainic acid at 10^{-3}M, DL-ibotenic acid at 10^{-3}M and L-quisqualic acid at 10^{-5} on FAN (frequently autoactive neurone) excitability. Ordinate scale, number of spike discharges per min. Abscissa scale, time course, each histogram is 1 min.

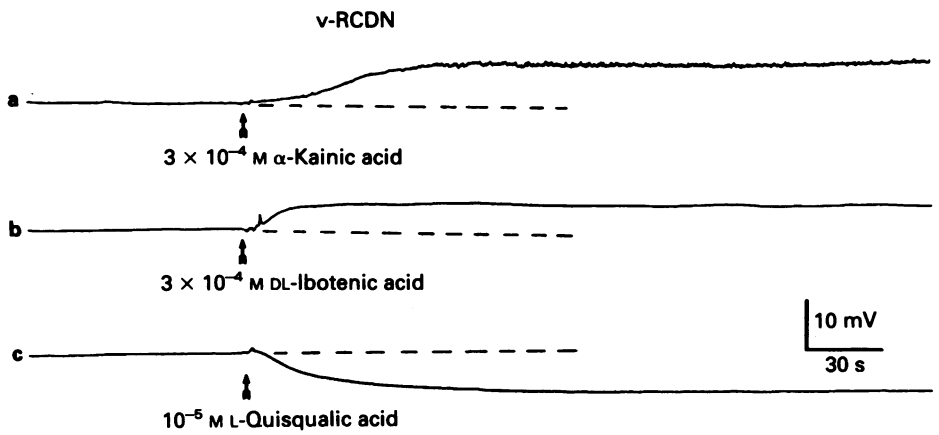

Figure 7 Effects of α-kainic acid at 3×10^{-4}M (a), DL-ibotenic acid at 3×10^{-4}M (b) and L-quisqualic acid at 10^{-5}M (c) on v-RCDN (ventral-right cerebral distinct neurone) membrane potential. The spike heights were cut electronically. Vertical bar, calibration (20 mV). Horizontal bar, time course (30 s).

Effects on threo-L-type neurones

The two neurones examined, FAN (frequently autoactive neurone) and v-RCDN (ventral-right cerebral distinct neurone), were more sensitive to threo-L-BHGA than to erythro-L-BHGA; both were inhibited by L-BHGA.

Figure 6 shows the effects of the three L-Glu analogues on FAN, with respect to the number of spike discharges. Among these substances, L-quisqualic acid produced the greatest inhibition which was similar to but more marked than that produced by L-BHGA; the MEC of L-quisqualic acid was 3×10^{-6}M (EPQ: 30). DL-Ibotenic acid was also inhibitory but less potent (EPQ: 0.1). On the other hand, α-kainic acid produced excitatory effects on FAN (EPQ: 0.3), which were opposite to those of L-BHGA. Erythro-L-tricholomic acid at 10^{-3}M displayed weak and unstable effects (EPQ: <0.1); domoic acid, α-allo-kainic acid and allo-L-GHGA at the same concentration had no effect.

Figure 7 shows the effects of the three L-Glu analogues on the membrane potential of v-RCDN. Of the substances examined, only L-quisqualic acid showed marked inhibitory effects (MEC: 3×10^{-6}M, EPQ: 3), similar to those of L-BHGA. On the other hand, α-kainic acid and DL-ibotenic acid were excitatory on v-RCDN; their MECs were: 3×10^{-5}M

(EPQ: 0.3) for α-kainic acid and 10^{-4}M (EPQ: 0.1) for DL-ibotenic acid. Domoic acid was also excitatory but less potent (EPQ: 0.1–0.03); erythro-L-tricholomic acid at 10^{-3}M had slight and biphasic (excitatory followed by inhibitory) effects (EPQ: 0.01); α-allo-kainic acid and allo-L-GHGA, at the same concentration, had almost no effect.

Discussion

In the present study on *Achatina fulica* Férussac, the effects of seven L-Glu analogues on six identifiable giant neurones, sensitive to L-BHGA, were examined. These neurones responded to L-BHGA but not to L-Glu, so it is proposed that the following four binding sites of L-BHGA to the receptors in the neuromembranes are needed to produce the effects: α-carboxyl group, α-amino group, β-hydroxyl group, and γ-carboxyl group.

All the substances examined possess the α-carboxyl group and the α-amino group (or the α-secondary amino group in some substances) in their structures. Therefore, the roles of the other two binding sites must be considered in order to explain the structures of these substances acting possibly on the L-BHGA receptors. The γ-carboxyl group acts possibly as (1) having the negative charge, or (2) the proton acceptor or donor. Possible roles of the β-hydroxyl group are: (1) the proton acceptor or donor, (2) filling the space between the substance and the receptor, (3) fixing the structure of the substance, or (4) increasing the negative charge (the acidity) of the substance.

Both α-kainic acid and domoic acid markedly excited d-RPLN and RAPN, which were also excited by L-BHGA. If these two substances having the γ-carboxyl group acted on the L-BHGA receptors of the two neurones, their 1′–2′ double bond should serve as the β-hydroxyl group of L-BHGA. The double bond can fulfil every possible role of the β-hydroxyl group described above, except for that of proton donor. Since the effects of domoic acid and α-kainic acid on the two neurones were almost equipotent, the 2′-substituent of domoic acid did not play any role, neither potentiating nor preventing the effects on the two neurones.

On the other hand, FAN and v-RCDN, inhibited by L-BHGA, were excited by α-kainic acid. Since these two neurones were excited by GABA (Ku & Takeuchi, 1983b; Ku *et al.*, 1985), the α-kainic acid appeared to be similar to GABA.

α-Allo-kainic acid, in contrast to α-kainic acid, had no effect on any of the neurones examined, indicating that 3- and 4-substituents of the substance are needed to be in *cis*-relationship to produce the effects.

Erythro-L-tricholomic acid had marked effects, similar to those of L-BHGA, on VIN and RAPN. If this substance acted on the L-BHGA receptors of these neurones, the 2-secondary amino group, having the negative charge, in its isoxazolidine ring should act as the γ-carboxyl group of L-BHGA, and the 1-oxygen or the 3-carbonyl group in the same ring should play the roles of the β-hydroxyl group of L-BHGA except as the proton donor.

The DL-compound of ibotenic acid was used in the present experiments, since its racemization occurs very easily. This substance showed marked effects similar to those of L-BHGA, on PON, VIN and RAPN. If DL-ibotenic acid acted as an agonist of L-BHGA on these neurones, the 2-secondary amino group, having the negative charge, in its isoxazoline ring, should act as the γ-carboxyl group, and the 1-oxygen or the 3-carbonyl group in the same ring should fulfil the roles of the β-hydroxyl group (not as the proton donor). On the other hand, DL-ibotenic acid excited v-RCDN, which was inhibited by L-BHGA and excited by GABA (Ku *et al.*, 1985).

L-Quisqualic acid showed marked effects, similar to those of L-BHGA, on all neurones examined. FAN and v-RCDN of the threo-type and RAPN of the combined type were more sensitive to this substance than the neurones in the erythro-L-type. If L-quisqualic acid acted on the L-BHGA receptors in these neurones, the 4-secondary amino group, having the negative charge, in its oxadiazolidine ring should act as the γ-carboxyl group; and 1-oxygen, 3-carbonyl or 5-carbonyl groups in the same ring should play the roles of the β-hydroxyl group. L-Quisqualic acid was generally much more effective than DL-ibotenic acid and erythro-L-tricholomic acid, due, at least in part, to the stronger negative charge of the 4-secondary amino group of L-quisqualic acid than those of the other two substances.

Allo-L-GHGA was almost without effect on any of the neurones examined, indicating that the β-hydroxyl group of L-BHGA cannot be replace with the γ-hydroxyl group so as to produce the effects.

The effects of the L-Glu analogues mentioned above on the L-Glu-sensitive excitable cells of the other invertebrate species have been studied by many investigators. Shinozaki & Shibuya (1974; 1976), Constanti & Nistri (1976) and Shinozaki & Ishida (1981) reported, using the crustacean neuromuscular junctions which were excited by L-Glu, that quisqualic acid and domoic acid were more excitatory than L-Glu; α-kainic acid was less excitatory than L-Glu; while ibotenic acid had little effect.

Walker *et al.*, (1971), Piggott *et al.* (1975) and Walker (1976) using giant neurones of the European garden snail, *Helix aspersa*, found that, with the neurone producing biphasic responses to L-Glu, quisqualic acid was much more excitatory than L-Glu, DL-ibotenic acid was equipotent with L-Glu, but α-kainic acid was less potent; with the L-Glu-excited

neurone, quisqualic acid was also much more excitatory than L-Glu, but DL-ibotenic acid was less potent, and α-kainic acid had almost no effect; and with the L-Glu-inhibited neurone, quisqualic acid was much more inhibitory than L-Glu, DL-ibotenic acid was equipotent to L-Glu but α-kainic acid had no effect. Consequently, the sensitivity of the L-Glu-inhibited neurone of *Helix aspersa* to the substances mentioned was especially comparable to that of PON, inhibited by L-BHGA, in *Achatina fulica* Férussac.

James *et al.* (1980a, b) reported much stronger excitatory effects of both α-kainic acid and quisqualic acid on the leech Retzius neurones than those of L-Glue. Roberts & Walker (1982) found that α-kainic acid and quisqualic acid showed marked excitatory effects on the L-Glu-excited neurones of the horseshoe crab, *Limulus polyphemus*; however, α-kainic acid had no effect, and quisqualic acid had effects opposite to those of L-Glu on the L-Glu-inhibited neurones.

As described above, the responses to the L-Glu analogues of the cells, sensitive either to L-BHGA or to L-Glu, were varied. It is not easy to point out clear differences between the responses of the former L-BHGA-sensitive neurones and those of the latter. On the other hand, L-quisqualic acid was obviously the most effective on these cells, and α-kainic acid and domoic acid acted mainly on the cells excited by either L-Glu or L-BHGA.

Extensive experiments on the electrophysiological anlyses of the effects of the L-Glu analogues on the L-BHGA-sensitive neurones will be conducted to demonstrate whether these L-Glu analogues act in the same manner as L-BHGA.

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