
Excitatory amino acids: physiological and pharmacological probes for neuroscience research

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Abstract. The *2S,3S,4S*-isomer (L-CCG-I) of 2-(carboxycyclopropyl)glycine (CCG) is a potent metabotropic glutamate receptor agonist. L-CCG-I depressed monosynaptic excitation in the newborn rat spinal motoneurone at low concentrations well below those causing postsynaptic depolarization. *2S,3R,4S*-CCG (L-CCG-IV) is a potent N-methyl-D-aspartate (NMDA)-type agonist. In cultured rat hippocampal neurones, L-CCG-IV caused marked increase in intracellular Ca^{2+} concentrations. 6-Carboxylated L-CCG-IV (DCG-IV), which is a tricarboxylated CCG derivative containing both chemical moieties of L-CCG-I and L-CCG-IV, depressed preferentially monosynaptic excitation of spinal reflexes in lower concentrations than L-CCG-I.

4-(2-Methoxyphenyl)-2-carboxy-3-pyrrolidineacetic acid (MFPA), which is the most potent kainoid yet described, is superior to acromelic acid A in causing depolarization of the newborn rat spinal motoneurone. In addition to MFPA, some non-kainoids demonstrated considerably high depolarizing activities. These new compounds would provide useful probes for neuroscience research.

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INTRODUCTION

L-Glutamate has been believed to be a major excitatory neurotransmitter at many synapses in vertebrates and invertebrates. The study of excitatory amino acids began with the investigation of their structure-activity relationships. At present, the pharmacological study of excitatory amino acids is progressing rapidly toward elucidation of the physiological roles of each receptor subtype. Central excitatory amino acid receptors now are most conveniently subdivided into five main classes (Monaghan et al. 1989); N-methyl-D-aspartate (NMDA)-, kainate-, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)- and L-2-amino-4-phosphonobutyric acid (L-AP4)-type receptors, and metabotropic glutamate receptors which are coupled to inositol-1,4,5-triphosphate (IP₃) and diacylglycerate turnover. NMDA receptors are competitively and effectively blocked by a number of ω -phosphono- α -amino acids, notably D-(-)-2-amino-4-phosphonovaleric acid (D-APV) and 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP). Some quinoxaline derivatives such as 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX) are blockers of both AMPA- and kainate-type receptors, but highly selective and potent antagonists at non-NMDA-type receptors are not yet discovered. The *1S,3R*-enantiomer (*1S,3R*-ACPD) of 1-aminocyclopentane-*trans*-1,3-dicarboxylate (*trans*-ACPD) was reported to be a selective agonist at metabotropic glutamate receptors (Irving et al. 1990). Although selective antagonists are essential for the pharmacological study of neurotransmitters, specific and potent glutamate agonists are still required to elucidate glutamate function further. As standard in such study, some compounds of natural origin, such as kainic acid, domoic acid, quinolinic acid and quisqualic acid, have played key roles as valuable pharmacological probes for neuroscience research on excitatory amino acids. In the present paper, neuropharmacological actions of some novel excitatory amino

acids of natural origin are described. These compounds provide useful information about the mechanism underlying glutamate's transmitter function.

2-(CARBOXYCYCLOPROPYL)-GLYCINE

The glutamate analog, 2-(carboxycyclopropyl)glycine (CCG), was isolated from the plants *Aesculus parviflora* and *Blighia sapida* (Fowden et al. 1969). CCG is a conformationally restricted analog of glutamate, in which the cyclopropyl group fixes the glutamate chain in an extended or folded form (Kurokawa et al. 1985, Yamanoi et al. 1988) and CCG has eight diastereomers (Shimamoto et al. 1991). Therefore, CCG would provide useful information about the interaction between the conformation of glutamate molecules and activation of each receptor subtype. Eight CCG stereoisomers (Fig. 1) demonstrated a large variety of depolarizing activities in the isolated newborn rat spinal cord (Shinozaki et al. 1989b, c). The depolarizing activity of the *2S,3S,4S*-isomer (L-CCG-I, an extended form) is higher than that of L-glutamate in the newborn rat spinal motoneurons, and is almost equal to that of *trans*-ACPD. Neither selective NMDA blockers nor CNQX depressed depolarizing responses to L-CCG-I, demonstrating a clear preference for non-NMDA, non-kainate and non-AMPA receptors. Depolarizing responses to L-CCG-I and *trans*-ACPD were markedly decreased by reducing the temperature of the perfusing fluid, clearly differentiating between ionotropic type agonists and the others (Ishida et al. 1990). These responses may suggest the participation of metabotropic glutamate receptors in the L-CCG-I-induced depolarization of newborn rat spinal motoneurons. Activation of metabotropic glutamate receptors blocks the slow Ca²⁺-dependent K⁺ conductance and increases the membrane excitability of neurones (Schoepp et al. 1990, Anwyl 1991, Baskys 1992). L-CCG-I induced oscillatory responses in *Xenopus* oocytes injected with rat brain mRNA (Ishida et al. 1990), and stimulated inositolphos-

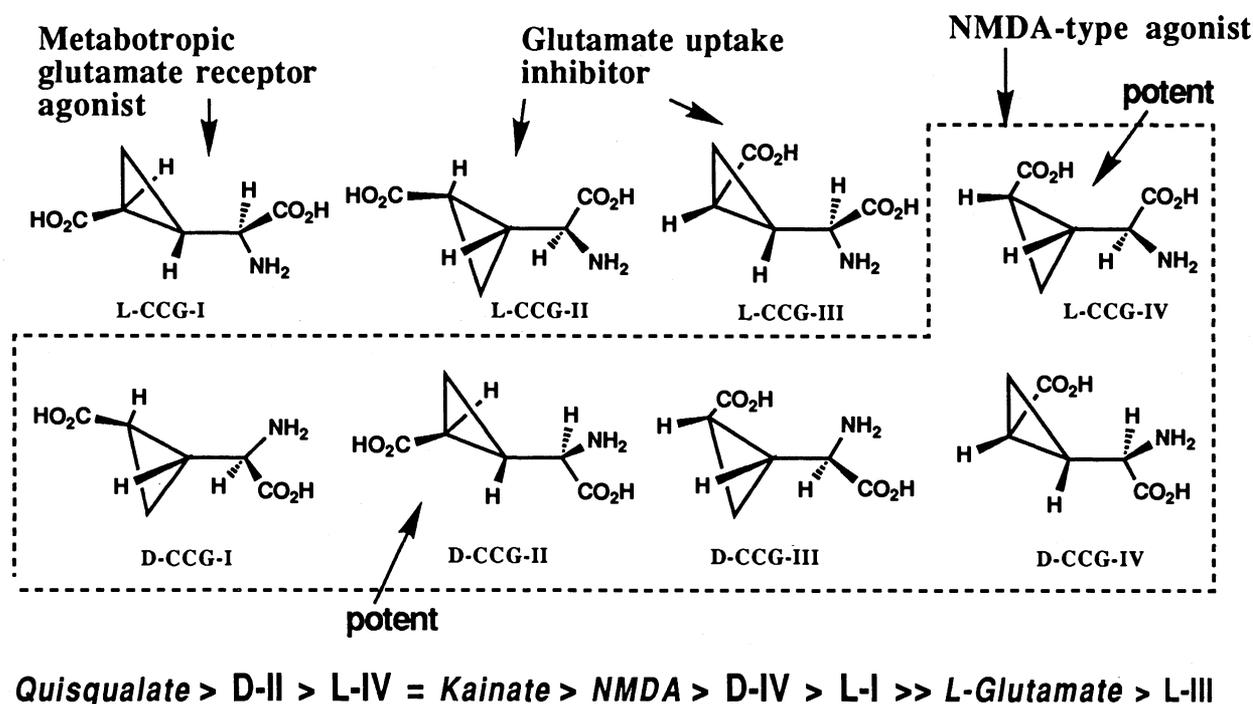


Fig. 1. Chemical structures of eight diastereomers of 2-(carboxycyclopropyl) glycine.

phate formation in rat hippocampal synaptoneuroosomes, demonstrating that L-CCG-I was a potent agonist for metabotropic glutamate receptors which are linked to GTP binding proteins (Nakagawa et al. 1990).

Metabotropic glutamate agonists block the excitatory synaptic transmission supported by the ionotropic glutamate receptor, and may therefore play a critical role in synaptic plasticity (Anwyll 1991). L-CCG-I preferentially reduced monosynaptic discharges induced by electrical stimulation of dorsal root fibres of newborn rats in low concentrations well below those causing postsynaptic depolarization (Fig. 2), though both mono- and poly-synaptic discharges were depressed in high concentrations (Shinozaki and Ishida 1992). This reduction of monosynaptic discharges was neither depressed by GABA antagonists such as picrotoxin and 2-hydroxysaclofen nor by any other pharmacological agents including glutamate antagonists. *1S,3R*-ACPD also depressed spinal reflexes, but it was accompanied by postsynaptic depolarization at all

times when monosynaptic excitation was decreased. Therefore, L-CCG-I seems to be functionally dissimilar to *1S,3R*-ACPD.

The *2S,3S,4R*-isomer (L-CCG-III, a folded form) caused marked potentiation of depolarizing responses to L-glutamate, D- and L- aspartate more effectively than L-(-)-*threo*-3-hydroxyaspartate, but did not affect the depolarization induced by kainic acid, quisqualic acid, NMDA and D-glutamate (Shinozaki et al. 1989c). There is clear evidence that this is due to inhibition of uptake of some excitatory amino acids (Kawai et al. 1992). In contrast to the actions of the *2S,3R,4S*-isomer (L-CCG-IV, a folded form), which is a potent NMDA agonist (see below), L-CCG-III demonstrated considerably lower depolarizing activity despite a folded common glutamate structure.

Among eight CCG stereoisomers, the *2R,3S,4S*-isomer (D-CCG-II) showed the highest depolarizing activity - about 5 times higher than that of NMDA in the newborn rat spinal cord - followed by L-CCG-IV (Shinozaki et al. 1989b). D-CCG-II and

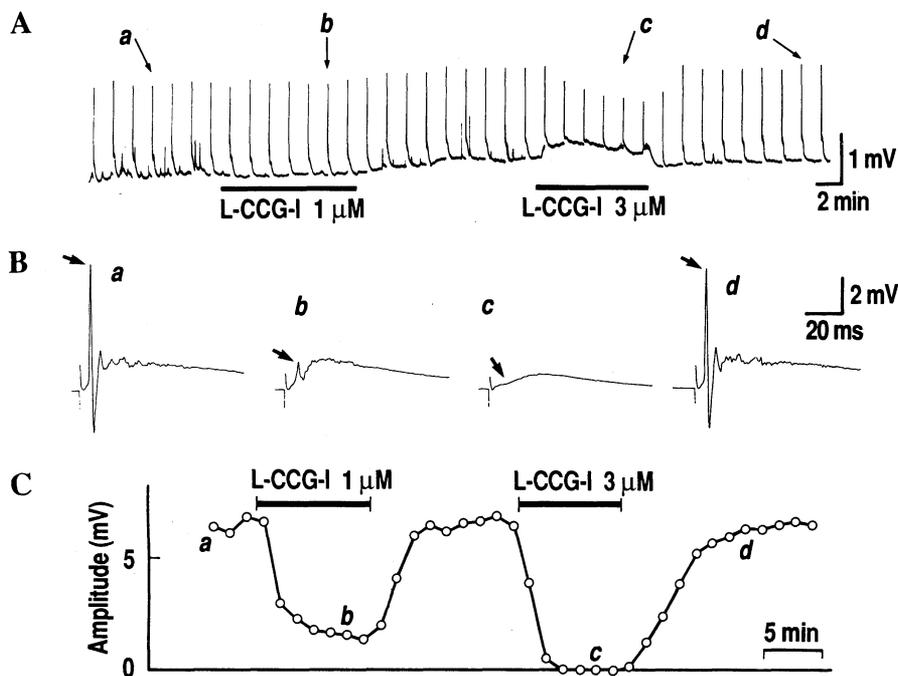


Fig. 2. Preferential depression of monosynaptic excitation of spinal reflexes by L-CCG-I in newborn rats. A, the upper trace represents low frequency components of spinal reflexes induced by the electrical stimulation of the dorsal root fibres. B, sample records of spinal reflexes on a X-Y recorder. a, b, c, d correspond to those in A. C, The time course of the peak amplitude of monosynaptic excitation in the presence of L-CCG-I.

L-CCG-IV are potent and specific NMDA agonists. This is the first time to find that an NMDA agonist with an L-configuration is more potent than NMDA. It is reasonable that L-CCG-IV causes an increase in intracellular Ca^{2+} concentrations, because Ca^{2+} influx into neurones through receptor ionchannels is one of characteristic features of function of NMDA receptors. Ca^{2+} may play a critical role in causing excitotoxic neuronal damage (Garthwaite and Garthwaite 1986, Choi 1987). L-CCG-IV increased intracellular Ca^{2+} concentrations much more markedly than NMDA in the cultured rat hippocampal neurone loaded with fura-2 (Shinozaki et al. 1991a, Kudo et al. 1991). The potency of L-CCG-IV to cause the increase was about 10 times higher than that of L-glutamate, and about 300 times higher than that of NMDA, and D-CCG-II and the 2*R*,3*S*,4*R*-isomer (D-CCG-IV) showed almost equal potencies to L-glutamate. Other CCGs were much less potent than NMDA. L-CCG-IV induced marked neuronal death in the CA1 area but not in the CA3 area of the rat hippocampus when injected intraventricularly, corresponding to the distribution of NMDA receptors. The carbon chain of glutamate can conform almost completely to that of CCG, and the structure of L-CCG-IV

closely mimics the folded conformation of L-glutamate, therefore, an active conformation of L-glutamate at NMDA receptors is considered to be a folded form.

The L-CCG-IV derivatives, 6*R*- and 6*S*-methoxymethyl-CCG and 6*R*-benzyloxymethyl-CCG, were much more potent than L-glutamate in causing depolarization of newborn rat spinal motoneurones. 6*R*-Methoxymethyl-CCG was approximately twice as potent as 6*S*-methoxymethyl-CCG and about 10 times more potent than 6*R*-benzyloxymethyl-CCG. The depolarization induced by 6*R*-derivatives was completely depressed by CNQX, but was almost insensitive to CPP (Ishida et al. 1991). The 6*R*-substituted CCG is a non-NMDA receptor agonist despite the fact that L-CCG-IV and its 6*R*-substituted compound have a folded common glutamate structure with the same configuration and conformation.

In the isolated dorsal root C-fibres of immature rats, kainic acid, domoic acid and L-glutamate produced considerable depolarization in a dose dependent manner, but quisqualic acid and AMPA caused only a slight depolarization at considerably high concentrations, and NMDA did not cause any depolarization even in high concentrations

(Agrawal and Evans 1986, Shinozaki et al. 1991b). Therefore, the dorsal root fibre of the immature rat spinal cord is practically useful for pharmacological classification of kainate receptor agonists. *6R*-Substituted CCGs caused a considerable depolarization in relatively low concentrations, suggesting the *6R*-substituted CCGs are kainate agonists (Ishida et al. 1991). L-CCG-IV caused slight depolarization of the dorsal root fibre at high concentrations. Significantly high concentrations of L-CCG-III induced only a slight depolarization, but other CCGs and *6S*-methoxymethyl-CCG, which showed NMDA-like depolarization in the spinal motoneurone, did not cause any depolarization of the dorsal root fibre even at high concentrations.

2-(4, 6-DICARBOXYCYCLOPROPYL) GLYCINE

DCG-IV (Fig. 3) is a tricarboxylated CCG derivative containing both chemical moieties of L-CCG-I and L-CCG-IV. DCG-IV caused NMDA-type depolarization of spinal motoneurons but its

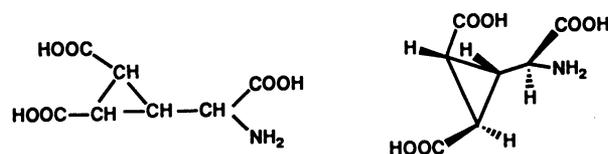


Fig. 3. Chemical structure of DCG-IV.

depolarizing activity became lower than that of L-CCG-IV or L-CCG-I, and the depolarization evoked by DCG-IV was completely depressed by selective NMDA antagonists, unlike the depolarization caused by L-CCG-I. As previously mentioned, L-CCG-I depressed preferentially monosynaptic excitation of the rat spinal reflexes. DCG-IV depressed monosynaptic excitation more effectively than L-CCG-I in low concentrations well below those causing postsynaptic NMDA-type depolarization. Baclofen, a GABA_B agonist, effectively depresses spinal reflexes in this preparation, but the activities of DCG-IV to depress the monosynaptic excitation was much higher than that of baclofen (Fig. 4). Picrotoxin, 2-hydroxysaclofen, bicuculline, and other pharmacological agents did

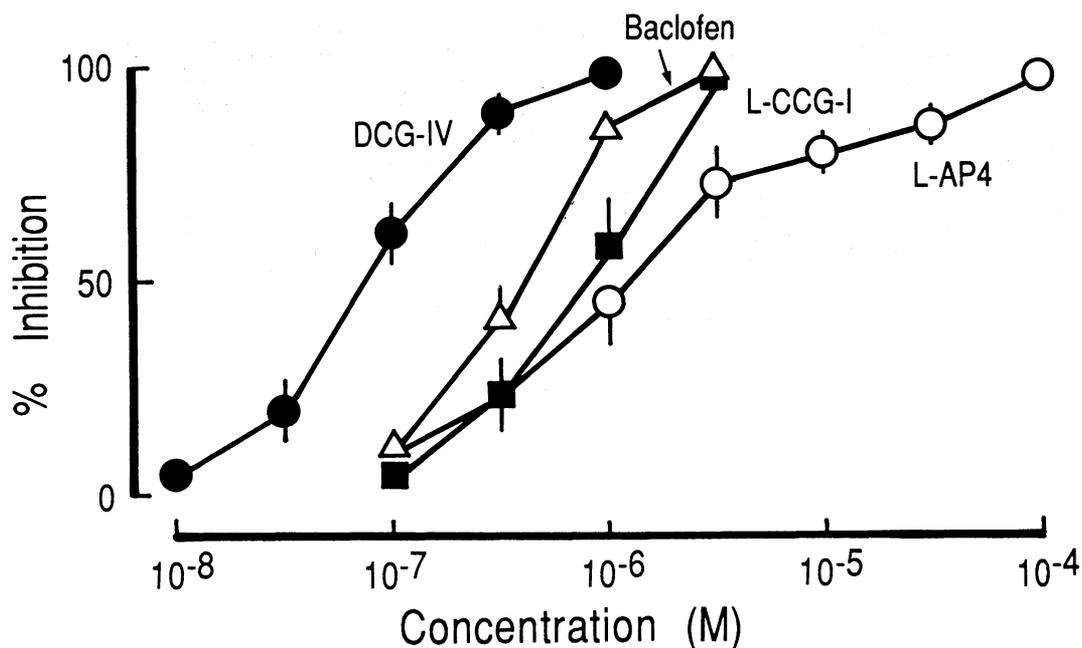


Fig. 4. Dose response curves for DCG-IV, baclofen, L-CCG-I and L-AP4 in inhibiting monosynaptic excitation of spinal reflexes induced by the electrical stimulation of the newborn rat dorsal root fibres. The inhibitory ratio of monosynaptic excitation was plotted against the concentration of each compound.

not block the reduction of monosynaptic reflexes induced by DCG-IV. Furthermore, DCG-IV itself did not depress any type of depolarization induced by excitatory amino acids. *1S,3R*-ACPD and L-AP4 also depressed monosynaptic discharges, but DCG-IV was functionally dissimilar to L-AP4, *1S,3R*-ACPD, L-CCG-I or baclofen. These inhibitory actions of DCG-IV on monosynaptic excitation seem to be due to activation of presynaptic receptors.

DCG-IV is expected to be a metabotropic glutamate receptor agonist from the structural similarity between DCG-IV and L-CCG-I. L-CCG-I stimulates inositolphosphate formation and decreased forskolin-stimulated cAMP content, however, DCG-IV does not increase phosphatidylinositol turnover, instead, preferentially inhibits forskolin-stimulated cAMP formation in the nerve cell at considerably lower concentration.

KAINATE AGONISTS

From the poisonous mushroom *Clitocybe acromelalga*, three kainoids (acromelic acid A, B and C) were isolated, which possess a constitutional moiety of kainic acid (Konno et al. 1983, 1988, Fushiya et al. 1990). Acromelic acid C is not an isomer of acromelic acid A or B, but has a structure of decarboxylated acromelic acid B. Acromelic acid A caused a more marked depolarization than kainic

acid or domoic acid in newborn rat spinal motoneurons (Ishida and Shinozaki 1988, Shinozaki et al. 1991b), and its electrophysiological properties are quite similar to those of kainic acid or domoic acid (Shinozaki 1988, Shinozaki 1992). Therefore, it is reasonable to assume that acromelic acids show excitotoxicity quite similar to kainic acid. However, a chain of abnormal behavioral signs induced by systemic administration of acromelic acid A to the rat was quite distinct from that of systemic kainate or domoate. The most pronounced changes were tonic extension of the hindlimbs, followed by flaccid paralysis and persistent spastic paraplegia (Shinozaki et al. 1989a, 1991b, Kwak et al. 1992b). These changes were never caused by kainic acid, instead, it caused severe limbic motor seizures. Systemic acromelic acid A causes selective neurone damage of small neurones in the lower spinal cord, but it does not cause lesions of spinal motoneurons (Kwak et al. 1992b).

Differences in behavioral signs and the distribution of neurone damage between acromelic acid A and kainic acid led us to search for novel kainoids which possibly revealed different kinds of neuropharmacological actions (Fig. 5) (Ishida and Shinozaki 1991, Shinozaki 1992). Some kainate derivatives caused depolarization much more effectively than kainic acid. In newborn rat spinal motoneurons, 4-(2-methoxyphenyl)-2-carboxy-3-pyrrolidineacetic acid (MFPA) was the most po-

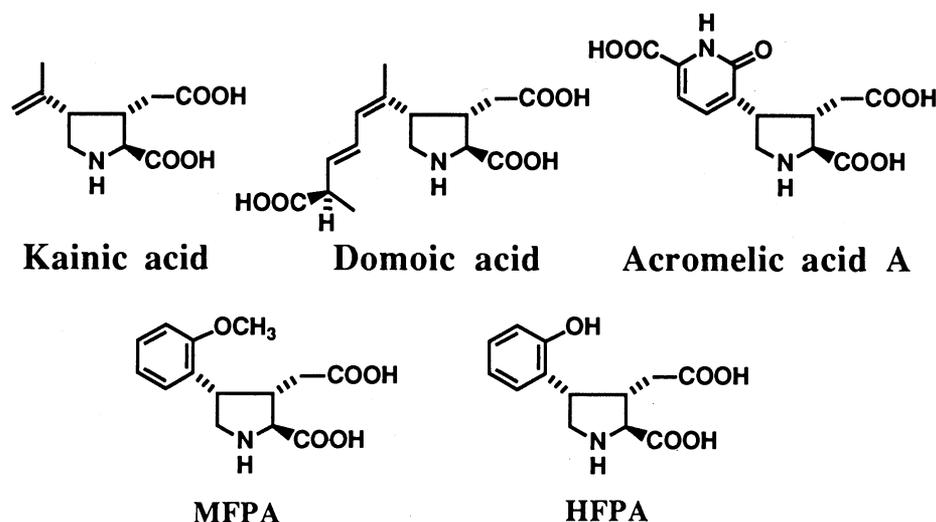


Fig. 5. Chemical structures of kainic acid, domoic acid, acromelic acid and newly synthesized potent kainoids.

tent among test samples in causing the depolarization. 4-(2-Hydroxyphenyl)-2-carboxy-3-pyrrolidineacetic acid (HFPA) also showed considerably high depolarizing activity. The rank order of depolarizing activities in motoneurons was MFPA > acromelic acid A > domoic acid \geq HFPA \geq acromelic acid B > HMPPA = CPPA = kainic acid > MPPA = FPA > CNOPA > MKPA. As acromelic acid A is so far the most potent among known excitatory amino acids, note that MFPA is more potent than acromelic acid A. The actions of MFPA and HFPA were effectively depressed by CNQX, but not by selective NMDA blockers. The rank order of above kainoids in the isolated dorsal root fibre is domoic acid > acromelic acid B > 5-bromowillardiine \geq MFPA > acromelic acid A > HFPA > kainic acid > CPPA > HMPPA > CNOPA = FPA \geq MKPA = L-glutamate. This order differed considerably from that obtained in spinal motoneurons. Cross desensitization between kainic acid and above kainoids suggests that these kainoids activated receptors in common with kainic acid in the dorsal root fibre.

The rank order of IC_{50} values for displacement with high affinity to [3H] kainate binding sites in the adult rat spinal cord was domoic acid > HFPA kainic acid = MFPA > quisqualate > acromelic acid B > L-glutamate > acromelic acid A \gg NMDA = AMPA, and that of [3H]AMPA was quisqualate > AMPA > MFPA = acromelic acid A > L-glutamate > HFPA > domoic acid > kainic acid \gg NMDA (Kwak et al. 1992a). MFPA and HFPA demonstrated high binding affinities for kainate receptors in the rat spinal cord, substantially comparable to

kainic acid or domoic acid. However, acromelic acids showed a considerably lower affinity for kainate receptors than kainic acid. Of particular interest, acromelic acid A and MFPA possessed valuable affinities for AMPA receptors. Smith (1992) recently reported that, in the absence of potassium thiocyanate, acromelic acid A is the most potent displacer of AMPA binding yet described, and he has presented that acromelic acid A distinguishes two kainate binding sites in rat brain synaptic plasma membranes. These compounds are expected to provide useful information for elucidating the diversity of kainate receptor function.

At this time the above mentioned discrepancy has no clear-cut explanation, though the idea of more than two types of kainate receptor subtypes is quite appealing. Systemic administration of MFPA induced interesting behavioral signs in rats, including both tonic extension of the hindlimbs and limbic seizures, which are characteristic of acromelic acid A and kainic acid, respectively. Multiplicity of kainate receptors also is proposed on the basis of electrophysiological evidence from cultured rat hippocampal neurones (Iino et al. 1990). In addition, recent studies on cDNA for glutamate receptors suggest the presence of a family of kainate receptor subunits with regional difference in the rat brain. Kainate or AMPA receptors may be composed of heterooligomeric subunits. The association of these subunits with one another would provide a number of different complexes, each exhibiting distinct electrophysiological properties such as preferential response to various kainate

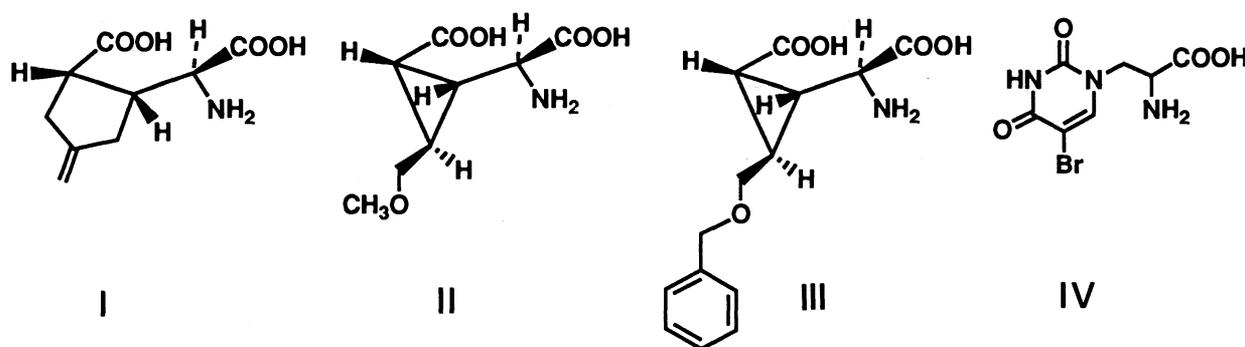


Fig. 6. Chemical structures of non-kainoids that activate kainate receptors.

agonists. Experimental results that non-kainoids activate kainate receptors led us to search for new non-kainoid kainate receptor agonists. So far 5-bromowillardiine has been known as a non-kainoids potent excitant of the dorsal root fibres. In fact, as mentioned above, 5-bromowillardiine was superior to MFPA or acromelic acid A in causing depolarization of the dorsal root fibre. The compounds shown in Fig. 6 demonstrated depolarizing activities in the dorsal root fibres with considerably high potency. In addition, the compound I in the figure demonstrated depolarizing activities comparable to those of kainic acid in the spinal motoneurone. Neuropharmacological actions of these compounds have not yet well documented in detail, but it will be expected that these compounds are useful for elucidating the diversity of kainate receptor function.

REFERENCES

- Anwyl R. (1991) The role of the metabotropic receptor in synaptic plasticity. *Trends Pharmacol. Sci.*, 12: 324-326.
- Agrawal S.G., Evans R.H. (1986) The primary afferent depolarizing action of kainate in the rat. *Br. J. Pharmacol.* 87: 345-355.
- Baskys A. (1992) Metabotropic receptors and 'slow' excitatory actions of glutamate agonists in the hippocampus. *Trends Neurosci.* 15: 92-96.
- Choi D.W. (1987) Ionic dependence of glutamate neurotoxicity in cortical cell culture. *J. Neurosci.* 7: 369-379.
- Fowden L., Smith A., Millington D.S., Sheppard R.C. (1969) Cyclopropane amino acids from *Aesculus* and *Blighia*. *Phytochem.* 8: 437-443.
- Fushiya S., Sato S., Kanazawa T., Kusano G., Nozoe S. (1990) Acromelic acid C, a new toxic constituent of *Clitocybe acromelalga*. *Tetrahedron Lett.* 31: 3901-3904.
- Garthwaite G., Garthwaite J. (1986) Neurotoxicity of excitatory amino acid receptor agonists in rat cerebellar slices. Dependence on calcium concentration. *Neurosci. Lett.* 66: 193-198.
- Iino M., Ozawa S., Tsuzuki K. (1990) Permeation of calcium through excitatory amino acid receptor channels in cultured rat hippocampal neurones. *J. Physiol.* 424: 151-156.
- Irving A.J., Schofield J.G., Watkins J.C., Sunter D.C., Collingridge G.L. (1990) 1S,3R-ACPD stimulates and L-AP3 blocks Ca^{2+} mobilization in rat cerebellar neurons. *Eur. J. Pharmacol.* 186: 363-365.
- Ishida M., Akagi H., Shimamoto K., Ohfuné Y., Shinozaki H. (1990) A potent metabotropic glutamate receptor agonist: electrophysiological actions of a conformationally restricted glutamate analogue in the rat spinal cord and *Xenopus* oocytes. *Brain Res.* 537: 311-314.
- Ishida M., Shimada Y., Shimamoto K., Ohfuné Y., Shinozaki H. (1991) Changes in preference for receptor subtypes of configurational variants of a glutamate analog: conversion from the NMDA-type to the non-NMDA type. *Brain Res.* 550: 152-156.
- Ishida M., Shinozaki, H. (1988) Acromelic acid is a much more potent excitant than kainic acid or domoic acid in the isolated rat spinal cord. *Brain Res.* 474: 386-389.
- Ishida M., Shinozaki H. (1991) Novel kainate derivatives: potent depolarizing actions on spinal motoneurons and dorsal root fibres in newborn rats. *Br. J. Pharmacol.* 104: 873-878.
- Kawai M., Horikawa Y., Ishihara T., Shimamoto K., Ohfuné Y. (1992) 2-(carboxycyclopropyl)glycine: binding, neurotoxicity and induction of intracellular free Ca^{2+} increase. *Eur. J. Pharmacol.* 211: 195-202.
- Konno K., Hashimoto K., Ohfuné Y., Shirahama H., Matsumoto T. (1988) Acromelic acid A and B. Potent neuroexcitatory amino acids isolated from *Clitocybe acromelalga*. *J. Am. Chem. Soc.* 110: 4807-4815.
- Konno K., Shirahama H., Matsumoto T. (1983) Isolation and structure of acromelic acid A and B. New kainoids of *Clitocybe acromelalga*. *Tetrahedron Lett.* 24: 939-942.
- Kudo Y., Akita K., Ishida M., Shinozaki H. (1991) A significant increase in intracellular Ca^{2+} concentration induced by (2S,3R,4S)-2-(carboxycyclopropyl)glycine, a new potent NMDA agonist, in cultured rat hippocampal neurons. *Brain Res.* 567: 342-345.
- Kurokawa N., Ohfuné Y. (1985) The palladium(II)-assisted synthesis of (\pm)- α -(methylenecyclopropyl)glycine and (\pm)-*trans*- α -(carboxycyclopropyl)glycine two bioactive amino acids. *Tetrahedron Lett.* 26: 83-84.
- Kwak S., Aizawa H., Ishida M., Shinozaki H. (1992a) New potent kainate derivatives: comparison of their affinity for [3H]kainate and [3H]AMPA binding sites. *Neurosci. Lett.* 139: 114-117.
- Kwak S., Aizawa H., Ishida M., Shinozaki H. (1992b) Acromelic acid, a novel kainate analogue, induces long-lasting paraparesis with selective degeneration of interneurons in the rat spinal cord. *Exp. Neurol.* 116: 145-155.
- Monaghan D.T., Bridges R.J., Cotman C.W. (1989) The excitatory amino acid receptors: the classes, pharmacology, and distinct properties in the function of the central nervous system. *Annu. Rev. Pharmacol. Toxicol.* 29: 365-402.
- Nakagawa Y., Saito K., Ishihara T., Ishida M., Shinozaki H. (1990) (2S,3S,4S)- α -(Carboxycyclopropyl)glycine is a novel agonist of metabotropic glutamate receptors. *Eur. J. Pharmacol.* 184: 205-206.
- Schoepp D., Bockaert I., Sladeczek F. (1990) Pharmacological and functional characteristics of metabotropic excitatory amino acid receptor. *Trends Pharmacol. Sci.* 11: 508-515.

- Shimamoto K., Ishida M., Shinozaki, H., Ohfuné Y. (1991) Synthesis of four diastereomeric L-2-(carboxycyclopropyl)glycines. Conformationally constrained L-glutamate analogues. *J. Org. Chem.* 56: 4167-4176.
- Shinozaki H. (1988) Pharmacology of the glutamate receptor. *Prog. Neurobiol.* 30: 399-435.
- Shinozaki H. (1992) Kainic acid receptor agonists. In: Excitatory amino acid receptors: design of agonists and antagonists. (Eds. P. Krogsgaard-Larsen and J.J. Hansen) Ellis Horwood p. 261-291.
- Shinozaki H., Ishida M. (1991) Recent advances in the study of glutamate receptor agonists. *Asia Pacific J. Pharmacol.* 6: 293-316.
- Shinozaki H., Ishida M. (1992) A metabotropic L-glutamate receptor agonist: pharmacological difference between rat central neurons and crayfish neuromuscular junctions. *Comp. Biochem. Physiol.* 103C: 13-17.
- Shinozaki H., Ishida M., Gotoh Y., Kwak S. (1989a) Specific lesion of rat spinal interneurons induced by systemic administration of acromelic acid, a new potent kainate analogue. *Brain Res.* 503: 330-333.
- Shinozaki H., Ishida M., Kudo Y. (1991a) Potent NMDA like actions of conformational variants of a glutamate analogue. In: NMDA Receptor Related Agents: Biochemistry, Pharmacology and Behaviour (Eds. T. Kameyama, T. Nabeshima and E.F. Domino) NPP Books, p. 247-260.
- Shinozaki H., Ishida M., Kwak S., Nakajima T. (1991b) Use of acromelic acid for production of rat spinal lesions. In: *Methods in Neuroscience*. Vol.7 (Ed. P.M. Conn) Academic Press, New York, p. 38-57.
- Shinozaki H., Ishida M., Shimamoto K., Ohfuné Y. (1989b) A conformationally restricted analogue of L-glutamate, the *threo*-folded form of L- α -(carboxycyclopropyl)glycine, activates the NMDA-type receptor more markedly than NMDA in the isolated rat spinal cord. *Brain Res.* 480: 355-359.
- Shinozaki H., Ishida M., Shimamoto K., Ohfuné Y. (1989c) Potent NMDA-like actions and marked potentiation of glutamate responses of conformational variants of a glutamate analogue in the rat spinal cord. *Brit. J. Pharmacol.* 98: 1213-1224.
- Smith A.L., McIlhinney R.A.J. (1992) Effects of acromelic acid A on the binding of [3 H]-kainic acid and [3 H]-AMPA to rat brain synaptic plasma membranes. *Br. J. Pharmacol.* 105: 83-86.
- Yamanoi K., Ohfuné Y., Watanabe K., Li P.N., Takeuchi H. (1988) Synthesis of *trans*- and *cis*- α -(carboxycyclopropyl)glycine, novel neuroinhibitory amino acids as L-glutamate analogue. *Tetrahedron Lett.* 29: 1181-1184.
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