Br. J. Pharmacol. (1993), **109**, 1169–1177

A novel metabotropic glutamate receptor agonist: marked depression of monosynaptic excitation in the newborn rat isolated spinal cord

M. Ishida, T. Saitoh, *K. Shimamoto, *Y. Ohfune & [1]H. Shinozaki

The Tokyo Metropolitan Institute of Medical Science, 3-18-22, Honkomagome, Bunkyo-ku, Tokyo 113, Japan and *Suntory Institute for Bioorganic Research, Shimamoto-cho, Mishima-gun, Osaka 618, Japan

1 Neuropharmacological actions of a novel metabotropic glutamate receptor agonist, (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV), were examined in the isolated spinal cord of the newborn rat, and compared with those of the established agonists of (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-I) or (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD).

2 At concentrations higher than 10 μM, DCG-IV caused a depolarization which was completely blocked by selective N-methyl-D-aspartate (NMDA) antagonists. The depolarization was pharmacologically quite different from that caused by L-CCG-I and (1S,3R)-ACPD.

3 DCG-IV reduced the monosynaptic excitation of motoneurones rather than polysynaptic discharges in the nanomolar range without causing postsynaptic depolarization of motoneurones. DCG-IV was more effective than L-CCG-I, (1S,3R)-ACPD or L-2-amino-4-phosphonobutanoic acid (L-AP4) in reducing the monosynaptic excitation of motoneurones.

4 DCG-IV (30 nM–1 μM) did not depress the depolarization induced by known excitatory amino acids in the newborn rat motoneurones, but depressed the baseline fluctuation of the potential derived from ventral roots. Therefore, DCG-IV seems to reduce preferentially transmitter release from primary afferent nerve terminals.

5 Depression of monosynaptic excitation caused by DCG-IV was not affected by any known pharmacological agents, including 2-amino-3-phosphonopropanoic acid (AP3), diazepam, 2-hydroxysaclofen, picrotoxin and strychnine.

6 DCG-IV has the potential of providing further useful information on the physiological function of metabotropic glutamate receptors.

Keywords: Excitatory amino acid; metabotropic glutamate receptor; presynaptic inhibition; spinal reflex; monosynaptic excitation; newborn rat spinal cord

Introduction

A metabotropic glutamate receptor, that is linked to the stimulation of phosphoinositide hydrolysis or the reduction of adenylcyclase activity, has recently been described (Sladeczek *et al.*, 1985; Nicoletti *et al.*, 1986; Sugiyama *et al.*, 1987; 1989; Palmer *et al.*, 1989; Tanabe *et al.*, 1992; Nakanishi, 1992). This glutamate receptor subtype in the mammalian central nervous system is activated by some kinds of glutamate analogues such as quisqualate, ibotenate, 1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) and the (2S,1'S,2'S)-isomer (L-CCG-I, previously reported as (2S,3S,-4S)-CCG) of 2-(carboxycyclopropyl)glycine (CCG) (Monaghan *et al.*, 1989; Sugiyama *et al.*, 1989; Schoepp *et al.*, 1990; Nakagawa *et al.*, 1990; Ishida *et al.*, 1990b; Shinozaki & Ishida, 1992; Porter *et al.*, 1992). (1S,3R)-ACPD and L-CCG-I appeared to be selective agonists for this receptor (Irving *et al.*, 1990; Shinozaki *et al.*, 1989b; Ishida *et al.*, 1990b) and others are non-selective agonists. The physiological effects of activation of metabotropic glutamate receptors have been examined in a number of brain regions (Schoepp *et al.*, 1990; Desai & Conn, 1991; Miller, 1991; Boss *et al.*, 1992). However, much less is known of the pharmacology of metabotropic glutamate receptors than is the case for ionotropic glutamate receptors which are characterized by their unique sensitivity to low concentrations of N-methyl-D-aspartic acid (NMDA), kainic acid and (RS)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid (AMPA) (Monaghan *et al.*, 1989; Simon, 1992; Krogsgaard-Larsen & Hansen, 1992). A major contributory factor has been lack of potent and selective agonists and antagonists of this class of L-glutamate receptors.

CCG is a conformationally restricted glutamate analogue (Shinozaki *et al.*, 1989b; Shinozaki & Ishida, 1991), and L-CCG-I is almost equal to (1S,3R)-ACPD and more potent than L-glutamate on a molar basis in causing a temperature-dependent depolarization of the newborn rat spinal motoneurone (Ishida *et al.*, 1990b). The depolarizing responses to L-CCG-I and (1S,3R)-ACPD were neither depressed by selective NMDA antagonists nor by a non-NMDA blocker, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), demonstrating a clear preference for non-NMDA, non-kainate and non-AMPA receptors (Shinozaki *et al.*, 1989b). L-CCG-I stimulates phosphoinositide hydrolysis in a concentration-dependent manner in rat hippocampal synaptoneurosomes (Nakagawa *et al.*, 1990), and it induces chloride-mediated oscillatory responses in *Xenopus* oocytes injected with rat brain mRNA in a manner quite similar to quisqualate or *trans*-ACPD (Ishida *et al.*, 1990b). Thus, L-CCG-I proved to be a potent agonist for metabotropic glutamate receptors. Recently, L-CCG-I was demonstrated to be a potent agonist for cloned 'mGluR2' glutamate receptors which are negatively coupled to adenylcyclase, although it stimulated cloned 'mGluR1' and 'mGluR4' glutamate receptors as well (Hayashi *et al.*, 1992).

It is known that (1S,3R)-ACPD depresses monosynaptic excitation of motoneurones in the newborn rat spinal cord (Sunter *et al.*, 1991). L-CCG-I also depresses it markedly rather than polysynaptic discharges in the low micromolar range (Ishida *et al.*, 1990a; Shinozaki & Ishida, 1992; 1993).

[1] Author for correspondence.

These effects have been considered to be due to their presynaptic actions. In addition to L-CCG-I, we recently found that another L-CCG-I analogue, (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV), depressed monosynaptic excitation at lower concentrations than those required by L-CCG-I (Ohfune *et al.*, 1993; Shinozaki & Ishida, 1993). DCG-IV possesses the glutamate skeleton of both L-CCG-I and the (2S,1'R,2'S)-isomer (L-CCG-IV) of CCG (Figure 1). L-CCG-IV is a potent agonist for NMDA receptors (Shinozaki *et al.*, 1989a,b; Kawai *et al.*, 1992) and is about 300 times more potent than NMDA in increasing the intracellular Ca^{2+} concentration in rat cultured hippocampal neurones (Kudo *et al.*, 1991; Shinozaki *et al.*, 1992), while L-CCG-IV is several times more potent than NMDA in causing a depolarization of spinal motoneurones of newborn rats. The structural similarity of DCG-IV to both L-CCG-I and L-CCG-IV might lead to the assumption that DCG-IV would show similar pharmacological properties to them, that is, DCG-IV would be expected to activate both NMDA receptors and metabotropic glutamate receptors simultaneously. In fact, DCG-IV reduced forskolin-induced adenosine 3':5'-cyclic monophosphate (cyclic AMP) formation in the rat hippocampal neurones about 10–15 times more effectively than (1S,3R)-ACPD, but did not stimulate phosphoinositide hydrolysis (Maruyama *et al.*, unpublished observation). DCG-IV definitely caused an NMDA-like depolarization but was much less active than NMDA or L-CCG-IV, and its pharmacological actions are not always similar to those of L-CCG-I or (1S,3R)-ACPD. Therefore, DCG-IV would be expected to be a metabotropic glutamate receptor agonist of the other type. In the present paper we describe neuropharmacological actions of DCG-IV in the isolated spinal cord of the newborn rat and compare them with those of L-CCG-I or (1S,3R)-ACPD.

Methods

The methods used for the electrophysiological experiments in the newborn rat isolated spinal cord were essentially similar to those described previously (Shinozaki *et al.*, 1989b). The spinal cords of 1–7 day-old Wistar rats were used for the experiments. Under ether anaesthesia, the spinal cord below the thoracic region was isolated, hemisected sagitally and placed in a 0.15 ml bath perfused at a fixed flow rate of 5–6 ml min^{-1} with artificial cerebrospinal fluid (ACSF) (in mM: NaCl 138.6, KCl 3.4, $CaCl_2$ 1.3, $MgCl_2$ 1.2, $NaHCO_3$ 21.0, NaH_2PO_4 0.58, glucose 10.0, pH 7.4) which was oxygenated with a gas mixture of 95% O_2 and 5% CO_2. In some experiments, isolated spinal cords were used without hemisection in order to record the contralateral ventral root potential. Tetrodotoxin (TTX, 0.5 μM) was sometimes added to ACSF in order to block spontaneous depolarization and indirect drug effects, and in some cases Mg^{2+}-free solution was used to test the depolarizing activity of glutamate analogues including NMDA-type agonists. The potential changes generated in the motoneurones were recorded extracellularly from the L4 or L5 ventral root with a suction electrode, and in some experiments the evoked reflex responses were recorded from the L4 or L5 ventral roots with suction electrodes when the ipsi- or contralateral root of the corresponding segment was stimulated with a suction electrode. Dorsal root potential (DRP) was recorded from a dorsal root (L4 or L5) adjacent to the stimulated root. All test compounds were applied to the preparation either by perfusion or by the brief-pulse injection into the perfusion system at a constant duration. The temperature of the perfusing fluid was kept at 27 ± 0.2°C.

Drugs

The following compounds were used: acetylsalicylate (Sigma), (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD, Tocris), (RS)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid (AMPA, Tocris), L-2-amino-4-phosphonobutanoic acid (L-AP4, Tocris), DL-2-amino-3-phosphonopropanoic acid (AP3, Tocris), baclofen (Sigma), bicuculline (Sigma), (RS)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP, Tocris), (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-I), (2S,1'R,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-IV), (RS)-4-carboxy-3-hydroxyphenylglycine (4C3H-PG, Tocris), 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX, Tocris), chlorzoxazone (Sigma), diazepam (Horizon inj, Yamanouchi), (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV), furosemide (Sigma), 2-hydroxysaclofen (Tocris), matrine (a gift from Dr M. Terada, Hamamatsu Univ. Shizuoka, Japan), N-methyl-D-aspartic acid (NMDA, Sigma), picrotoxin (Tokyo Kasei), piperazine hydrochloride (Sigma), pertussis toxin (List Biol. Lab.), sodium L-glutamate mono (Wako), strychnine hemisulphate (Sigma), tetrodotoxin (TTX, Seikagakukogyo) and theanine (a gift from Dr M. Nakagawa, National Research Institute of Tea, Shizuoka, Japan). The carbon numbers of CCG derivatives previously reported were renumbered according to the IUPAC nomenclature in the present paper.

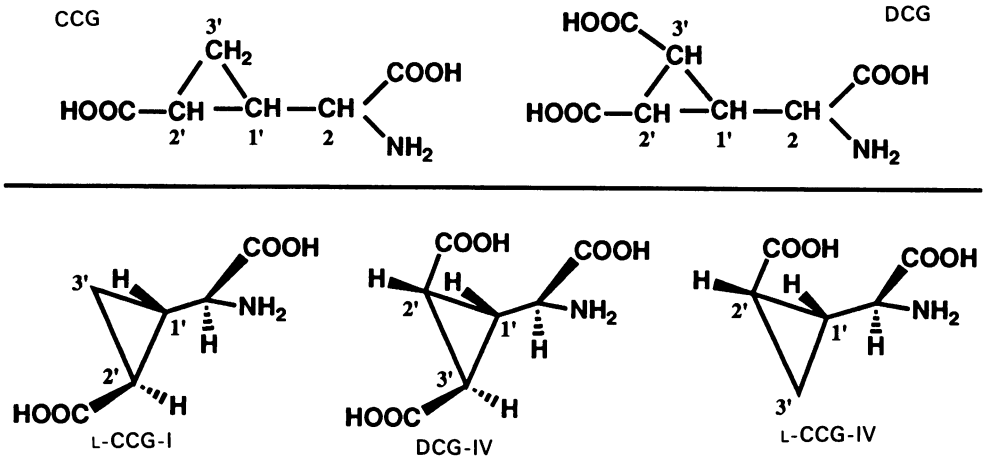

Figure 1 Plane chemical structure of 2-(carboxycyclopropyl)glycine (CCG) and 2-(2,3-dicarboxycylcopropyl)glycine (DCG), and stereochemical display of (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-I), (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl)-glycine (DCG-IV) and (2S,1'R,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-IV). The carbons 1', 2' and 3', previously numbered as 3, 4 and 6, respectively, were renumbered according to the IUPAC nomenclature.

Results

Depolarization of motoneurones induced by DCG-IV

The potential changes generated in spinal motoneurones of newborn rats were recorded extracellularly from the L4 or L5 ventral root of the hemisected spinal cord. When L-CCG-I, (1S,3R)-ACPD and DCG-IV were added to the TTX (0.5 μM) containing and Mg^{2+}-free bathing solution for a period of 30 s, they caused a depolarization of spinal motoneurones in a concentration-dependent manner. Their threshold concentrations for depolarization were less than 10 μM. L-CCG-I and (1S,3R)-ACPD showed slightly slower responses (the prolonged time course of action) than those to L-glutamate. The depolarizing activity of DCG-IV was about 20 times higher than that of L-glutamate on a molar basis, but lower than that of NMDA or L-CCG-IV. Figure 2 shows the depolarizing responses to L-glutamate, NMDA, AMPA, DCG-IV, L-CCG-I and (1S,3R)-ACPD in the presence and absence of (RS)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) and CNQX. The depolarization induced by L-CCG-I or (1S,3R)-ACPD was only slightly reduced by CPP, and the pharmacological properties of the depolarization seemed quite similar (Shinozaki *et al.*, 1989b; Ishida *et al.*, 1990b). On the other hand, the depolarizaiton induced by DCG-IV (20 μM) was completely blocked by CPP (10–50 μM), suggesting that DCG-IV activated NMDA receptors, as might be anticipated from the structural similarity between DCG-IV and L-CCG-IV (a potent NMDA agonist). It seems likely that the glutamate folded skeleton in the molecule of DCG-IV, which is quite similar to that of L-CCG-IV, contributes directly to the activation of NMDA receptors.

When the potential changes were recorded from the L4 or L5 dorsal root with a suction electrode, L-CCG-I, (1S,3R)-ACPD and DCG-IV caused depolarizations that were markedly smaller than those of ventral roots. Their threshold concentrations for depolarization were about 10 μM (TTX 0.5 μM containing, Mg^{2+}-free solution), demonstrating no significant difference in their threshold concentrations between the potentials from ventral and dorsal roots. The depolarization induced by DCG-IV completely disappeared in the presence of CPP (50 μM), but others were not reduced even in the combined treatment with CPP (50 μM) and CNQX (50 μM).

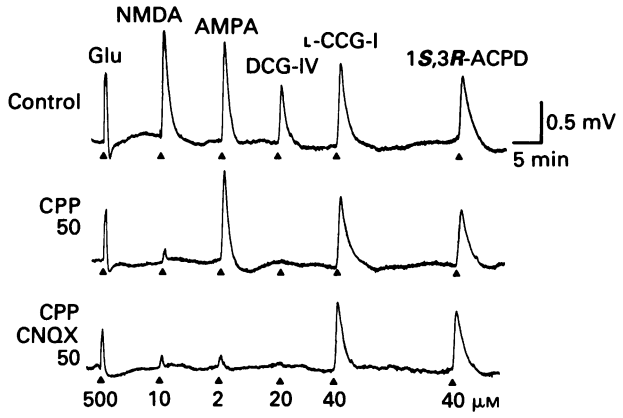

Figure 2 Sample records of the depolarizing responses to (2S,1'R,-2'R,3'R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV), (2S,1'S,2'S)-2-(carbodycyclopropyl)glycine (L-CCG-I), (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD) and representative excitatory amino acids in the newborn rat spinal motoneurone. Test samples were added to the tetrodotoxin (0.5 μM) containing and Mg^{2+}-free solution at triangles for a period of 30 s in the absence (control) and presence of (RS)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) and/or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Numbers show the concentration (μM) of the test samples. Glu: L-glutamate, NMDA: N-methyl-D-aspartate, AMPA: (RS)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid.

One of the pronounced features of the depolarization induced by the prolonged application of L-CCG-I and (1S,3R)-ACPD was a desensitization-like phenomenon, which was not observed in the case of DCG-IV. When L-CCG-I or (1S,3R)-ACPD was added to the bathing fluid (TTX 0.5 μM containing, Mg^{2+}-free) for 2 min in various concentrations, the depolarization induced by L-CCG-I or (1S,3R)-ACPD was not maintained but decreased during its application in a concentration-dependent manner (Figure 3a). On the other hand, DCG-IV and representative ionotropic glutamate receptor agonists, such as kainic acid, NMDA and AMPA, induced slight desensitization only when amplitudes of depolarizing responses were significantly large, in contrast to L-CCG-I and (1S,3R)-ACPD (data were not shown). The desensitization-like phenomenon observed here was also demonstrated when L-CCG-I and (1S,3R)-ACPD were repeatedly or alternately applied at short intervals. This desensitization-like action crossed between L-CCG-I and (1S,3R)-ACPD (Figure 3b). These time-dependent actions were not observed with repetitive application of NMDA, kainate and AMPA.

It has been reported that DL-2-amino-3-phosphonopropanoic acid (AP3), L-2-amino-4-phosphonobutanoic acid (L-AP4) and (S)-4-carboxy-3-hydroxyphenyl-glycine ((S)-4C3H-PG) inhibit depolarizing responses to *trans*- or (1S,3R)-ACPD in various preparations (Schoepp *et al.*, 1990; Irving *et al.*, 1990; Jones *et al.*, 1992). AP3 and L-AP4 induced slight depolarization of the newborn rat spinal motoneurone at a concentration greater than 0.3 mM, but neither AP3 nor L-AP4 reduced depolarizing responses to L-glutamate (1 mM, 10 s application), L-CCG-I (0.1 mM, 10 s), quisqualate (2 μM, 10 s), (1S,3R)-ACPD (0.1 mM, 10 s) and DCG-IV (50 μM, 10 s) even at higher concentrations than the threshold for depolarization of motoneurones. 4C3H-PG reduced the amplitude of depolarizing responses to L-CCG-I (40 μM, 30 s) and (1S,3R)-ACPD (40 μM, 30 s) to about half at a high concentration (300 μM) without causing depolarization of motoneurones in the TTX-containing solution.

Suppression of fluctuation of ventral root potentials

When the spinal preparation was perfused with the TTX-free ACSF, the base line of the potential derived from the L4 or L5 ventral root fluctuated in a range of about 0.5 mV, probably due to spontaneous discharges of motoneurones through the release of neurotransmitters. Low concentrations of L-CCG-I (0.1–1 μM, 10 min application) or DCG-IV (30–100 nM, 10 min application) almost completely reduced the baseline fluctuation without causing detectable depolarization of spinal motoneurones (Figure 4a), demonstrating the block of transmitter release from primary afferent nerve terminals. The recovery from the action of L-CCG-I was relatively rapid

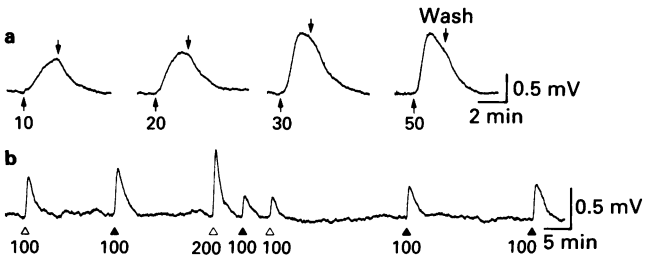

Figure 3 The desensitization-like responses revealed by (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-I) and (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD) in the newborn rat motoneurone. Test samples were added to the tetrodotoxin (0.5 μM)-containing and Mg^{2+}-free solution for a period indicated by arrows. Numbers show the concentration (μM) of the sample. (a) Depolarizing responses to L-CCG-I (2 min application, at intervals of 30 min); (b) 10 s application. (△) L-CCG-I; (▲) (1S,3R)-ACPD.

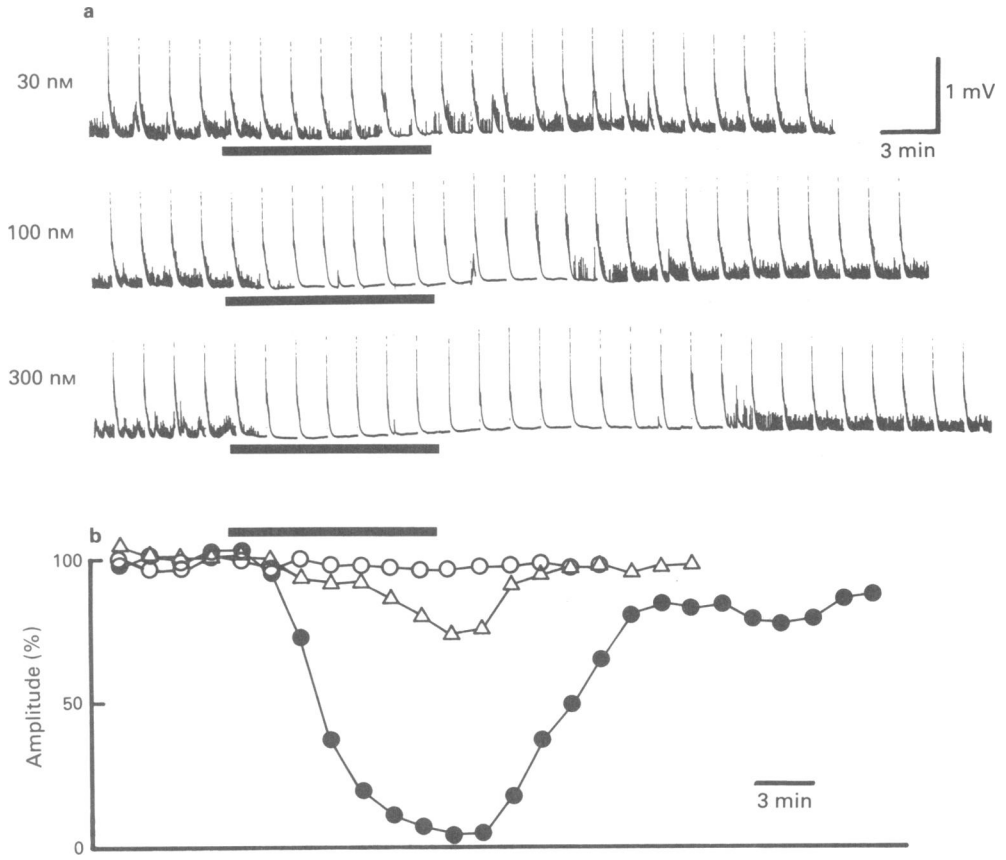

Figure 4 (a) Sample records of spinal reflexes evoked by electrical stimulation of L5 dorsal root of the isolated spinal cord of newborn rats (7-day-old). The potential changes were recorded from ipsilateral L5 ventral root. (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) was added to the perfusing fluid at a concentration of 30 (upper trace), 100 (middle trace) and 300 nM (lower trace) for a period indicated by horizontal bars. The fluctuation of the base line reduced in a dose-dependent manner. In these traces, the monosynaptic component of spinal reflexes were not shown because of the frequency response of the pen-writer (see b). The larger deflections represent polysynaptic discharges. (b) Time courses of dose-dependent reduction of the monosynaptic component of spinal reflexes in the presence of DCG-IV. Data were obtained from the same preparation as that in (a). DCG-IV 30 (○), 100 (△) and 300 (●) nM.

after washing the preparation (within about 5 min), but that of DCG-IV was slightly slower than that of L-CCG-I. (1S,3R)-ACPD (0.3–1 μM) did not reduce the fluctuation of the base line at concentrations less than the threshold concentration for depolarization of motoneurones, but rather slightly increased it.

Depression of spinal reflexes

The spinal reflex was repetitively evoked at intervals of 90 s by electrical stimulation of the L4 or L5 dorsal root of the newborn rat. Single shocks were delivered to dorsal root through stimulating electrodes. The potential changes (DR-VRP) were recorded from the ipsilateral ventral roots of the corresponding segment. The DR-VRP was reversibly depressed by DCG-IV at markedly low concentrations in a similar manner to L-CCG-I, and in particular, the DR-VRP monosynaptic component was preferentially decreased by DCG-IV at the nanomolar range, which was much lower than the threshold concentration (about 10 μM in case of TTX-free ACSF) for depolarization of motoneurones without reducing polysynaptic discharges (Figure 4b). In the presence of 0.1–0.3 μM DCG-IV (10 min application), the DR-VRP monosynaptic component almost disappeared with no associated depolarization of the postsynaptic membrane of motoneurones, but polysynaptic discharges were hardly affected. However, polysynaptic components of the DR-VRP were gradually decreased as the concentration was increased and at 5 μM (10 min application), DCG-IV completely reduced the polysynaptic component of the DR-VRP.

In order to test antagonistic actions of DCG-IV against various excitatory amino acids, AMPA (2 μM), NMDA (10 μM), kainic acid (3 μM), (1S,3R)-ACPD (40 μM) and L-CCG-I (40 μM) were added to the TTX-containing and Mg^{2+}-free ACSF. DCG-IV (30 nM–1 μM) did not reduce the depolarization of motoneurones thus induced, suggesting that DCG-IV does not affect depolarizing responses to excitatory amino acids including agonists for metabotropic glutamate receptors. The DR-VRP monosynaptic component is effectively reduced by non-NMDA antagonists, but not by selective NMDA blockers (Jahr & Yoshioka, 1986). As mentioned above, DCG-IV depressed the DR-VRP monosynaptic component at markedly low concentrations which did not cause depolarization of motoneurones. Even in a high concentration (50 μM), CPP did not reduce the DR-VRP monosynaptic component. On the other hand, DCG-IV depressed the DR-VRP monosynaptic component in the presence of high concentrations of CPP, suggesting that the inhibitory action of DCG-IV on spinal reflexes was neither the result of activation of NMDA receptors nor antagonism against neurotransmitters on postsynaptic membranes. It is likely that DCG-IV stimulates presynaptic events to depress the monosynaptic excitation of motoneurones.

L-AP4 has been regarded as one of representative agonists for an ionotropic glutamate receptor (Koerner & Cotman, 1981; Yamamoto *et al.*, 1983; Monaghan *et al.*, 1989), and L-AP4 is known to suppress the DR-VRP monosynaptic component in the spinal cords of both cats (Davies & Watkins, 1982) and immature rats (Evans *et al.*, 1982) at relatively low concentrations. Current information on

presynaptic L-AP4 receptors is also consistent with a second messenger mechanism in which L-AP4 blocks activation of voltage-sensitive Ca^{2+} channels (Koerner & Johnson, 1992). Furthermore, L-AP4 is a potent agonist for the cloned 'mGluR4' glutamate receptor expressed in Chinese hamster ovary cells, which is negatively coupled with adenyl cyclase (Nakanishi, 1992). So, the inhibitory actions of DCG-IV on the DR-VRP were compared with those of L-AP4, L-CCG-I and (1S,3R)-ACPD (Figure 5). L-AP4 (0.3–100 μM, 10 min application) depressed the fluctuation of the base line and the DR-VRP monosynaptic component without causing postsynaptic depolarization of the motoneurone as well as L-CCG-I or DCG-IV, but the actions were slightly less intense than those of L-CCG-I, and markedly less than those of DCG-IV on a molar basis. The threshold concentration of L-AP4 for suppression of the DR-VRP monosynaptic component was about 0.3 μM, which was similar to that of L-CCG-I but considerably higher than that of DCG-IV, but at concentrations of 30–100 μM, both monosynaptic excitation and polysynaptic discharges still remained in lessened amplitudes, suggesting the difference in action between DCG-IV and L-AP4. The threshold concentrations of DCG-IV, L-CCG-I and L-AP4 for decreasing polysynaptic discharges were about 0.3, 1 and 1 μM, respectively. (1S,3R)-ACPD (1–10 μM, 10 min application) depressed both mono- and polysynaptic components of the DR-VRP almost in parallel, with depolarization of motoneurones. Thus, the action of DCG-IV is similar to that of L-CCG-I in that they depress preferentially the monosynaptic component of the DR-VRP at lower concentrations, although there is a large variation of their effective concentration-range.

Effect of some pharmacological agents

For the pharmacological characterization of DCG-IV, the influence of picrotoxin (20 μM), bicuculline (3 μM), 2-hydroxysaclofen (100 μM), strychnine (1 μM), diazepam (10–100 μM), AP3 (100–300 μM), 4C3H-PG (10–300 μM), theanine (1 mM) (Shinozaki & Ishida, 1978), acetylsalicylate (100 μM), piperazine (100 μM), furosemide (100 μM), chlorzoxazone (100 μM) and matrin (0.5 mM) (Ishida & Shinozaki, 1984) were examined on the reduction of the DR-VRP monosynaptic component caused by DCG-IV. At concentrations greater than 30 μM, 4C3H-PG itself depressed both mono- and polysynaptic components of spinal reflexes (see Jones *et al.*, 1992); therefore, the action was examined at a concentration of 10 μM. Any tested compounds in the present experiments (10 min application) had no influence on the action of DCG-

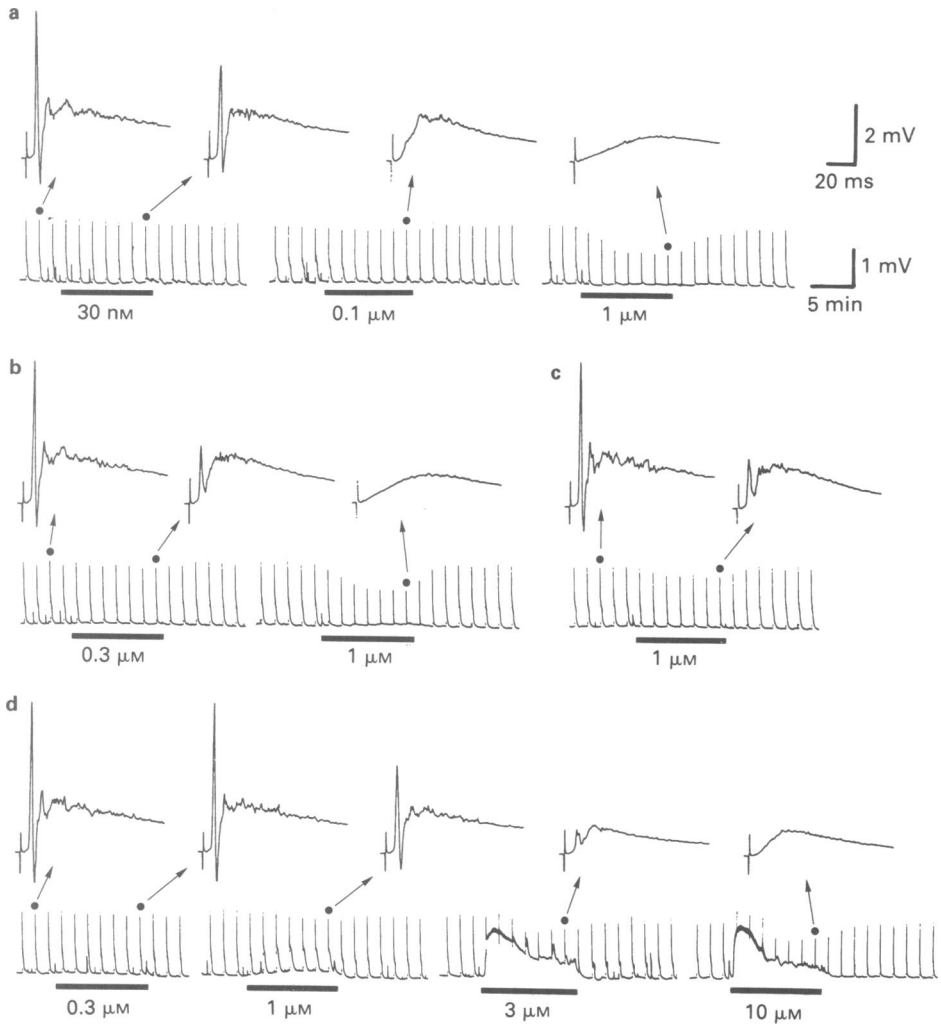

Figure 5 Sample records of spinal reflexes repetitively evoked at intervals of 90 s by electrical stimulation of the L5 dorsal root of the isolated spinal cord of newborn rats (1-day-old). The potential changes were recorded from the ipsilateral L5 ventral root. Upper chart: records on an X-Y recorder; Lower chart: records on a pen-writer. Upward fluctuation represents amplitudes of polysynaptic discharges. The following compounds were added to the perfusing fluid (tetrodotoxin-free, Mg^{2+} (1.2 mM) containing) for a period indicated by horizontal lines: (a) (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV); (b) (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-I); (c) L-2-amino-4-phosphonobutanoic acid (L-AP4); (d) (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD).

IV. CNQX itself (0.3–10 μM) reduced effectively the DR-VRP monosynaptic component, so the action of CNQX was not examined. In some experiments where the preparation was treated with pertussis toxin (3 μM) for 3 h, the DR-VRP monosynaptic component disappeared but the polysynaptic discharges still remained; therefore, it was impossible to examine the effect of DCG-IV on the DR-VRP monosynaptic component. However, in such cases, L-CCG-I (100 μM, 10 s application) still caused a depolarization, suggesting that the toxin failed to enter into the nerve cell.

DCG-IV and L-CCG-I have a common glutamate skeleton which takes an extended form that is supposed to activate metabotropic glutamate receptors (Ishida *et al.*, 1990b). If DCG-IV and L-CCG-I act on a common receptor, the action of DCG-IV might be expected to be attenuated or modified by L-CCG-I, although their effective concentration-range was different in depressing the monosynaptic component of spinal reflexes. In the presence of DCG-IV (0.3 μM), the DR-VRP monosynaptic component almost disappeared, and L-CCG-I (0.3 μM) reduced its amplitude to about 60% of the control. When both L-CCG-I (0.3 μM) and DCG-IV (0.1 μM) were added to the ACSF, the DR-VRP monosynaptic component disappeared entirely, suggesting that L-CCG-I did not antagonize against DCG-IV on the monosynaptic excitation. Furthermore, during combined treatment with a relatively low concentration of L-CCG-I (0.3 μM) and DCG-IV (0.03 μM), the DR-VRP monosynaptic component was reduced in an almost additive manner.

Comparison with baclofen

The above experiments show that the action of DCG-IV is not mediated by GABAergic systems, because picrotoxin, bicuculline and 2-hydroxysaclofen did not affect the action of DCG-IV. However, it is of great interest to compare the neuropharmacological actions of baclofen and DCG-IV, because both DCG-IV and baclofen reduce forskolin-induced cyclic AMP formation in nerve cells (Hill, 1985; Karbon & Enna, 1985), and baclofen is so far the most potent amino acid in depressing spinal reflexes of the newborn rat spinal preparation (Akagi & Yanagisawa, 1987). The L4 or L5 ventral root potential alternatively evoked by electrical stimulation of the ipsi- and contralateral dorsal root fibre of the corresponding segment (ipsi- and contralateral DR-VRP), and the dorsal root potential (DR-DRP) derived from the dorsal root adjacent to the stimulated root were recorded in the absence and presence of DCG-IV, baclofen and L-AP4. DCG-IV was more potent than baclofen in reducing the monosynaptic component of the ipsilateral DR-VRP (Figure 6a). On the other hand, DCG-IV was less active than baclofen in reducing the DR-DRP, although there was no difference in the effective concentration-range of baclofen between the monosynaptic component of the ipsilateral DR-VRP and the DR-DRP (Figure 6b). When the potential was recorded from the contralateral ventral root of the same segment as that of the stimulated dorsal root, fast and slow components of reflexes were observed. The slow component (contralateral slow DR-VRP) had a time-to-peak of 2–5 s and lasted 20–30 s. Baclofen depressed the contralateral slow DR-VRP at much lower concentrations than those required for depression of the monosynaptic component of the ipsilateral DR-VRP (Figure 7). Similar results were obtained by Akagi & Yanagisawa (1987). DCG-IV also reduced the contralateral slow DR-VRP at slightly higher concentrations than those required for the reduction of the monosynaptic component of the ipsilateral DR-VRP. These results suggest that the distribution of $GABA_B$ receptors in the newborn rat spinal cord is different from that of the DCG-IV-sensitive receptors. L-AP4 also blocks the contralateral slow DR-VRP more effectively than DCG-IV. The action of (1S,3R)-ACPD and L-CCG-I was slightly less potent than that of DCG-IV.

When the ED_{50} value for decreasing the ipsilateral DR-VRP monosynaptic component was compared between

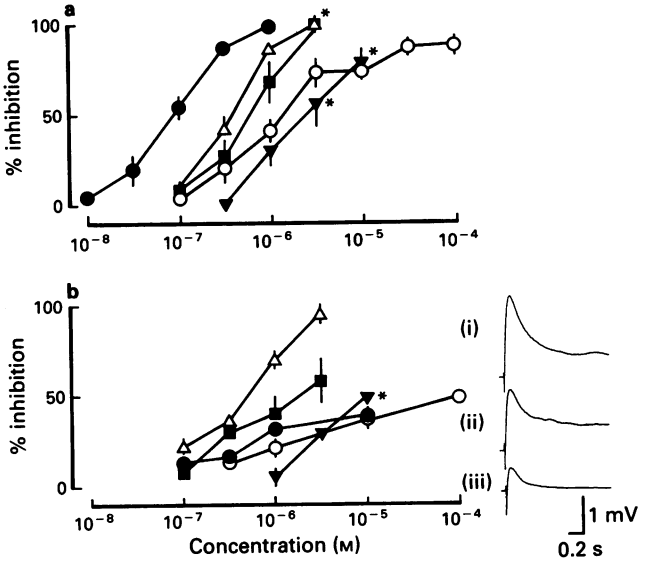

Figure 6 (a) The potential changes generated in the mononeurones were recorded extracellularly from the L4 ventral root with a suction electrode, and an ipsilateral dorsal root was electrically stimulated (10 V, 300 μs duration, 90 s interval) with a suction electrode. Dose-response curves for (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl)-glycine (DCG-IV) (●); baclofen (△); (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-I) (■); L-2-amino-4-phosphonobutanoic acid (L-AP4) (○) and (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD) (▼) on the reduction of the monosynaptic component of spinal reflexes of the newborn rat (3 or 4-day-old). Percentage inhibition of amplitudes of monosynaptic components of spinal reflexes was plotted against the concentrations. Vertical bars represent s.e.mean (*n* at least 4). *Concentrations which caused depolarization of motoneurones. (b) Dose-response curves for baclofen (△), L-CGG-I (■), DCG-IV (●), L-AP4 (○) and (1S,3R)-ACPD (▼) on the reduction of dorsal root potentials (DR-DRP). Vertical bars represent s.e.mean (*n* at least 4). The potential changes (DR-DRP) generated in the L4 dorsal root were recorded extracellularly with a suction electrode when the ipsilateral L5 dorsal root or an adjacent segment was electrically stimulated (10 V, 300 μs duration, 90 s interval) with a suction electrode. (i) control, (ii) DCG-IV (1 μM), (iii) baclofen (1 μM). *Concentrations which caused depolarization of dorsal roots.

1- and 7-day-old rats, the value increased to about three times with the lapse of days after birth in the case of DCG-IV, L-CCG-I, (1S,3R)-ACPD and L-AP4, suggesting that receptors sensitive to these amino acids decrease in number or activity immediately after birth by developmental regulation. However, it was not until the rat was at least 7 days old that the sensitivity to baclofen was changed markedly.

Discussion

The interactions of glutamate with its various receptor subtypes are not well understood with respect to their preference for particular conformers of the neurotransmitter molecules. (1S,3R)-ACPD and L-CCG-I, which have been established as potent agonists for metabotropic glutamate receptors (Nakagawa *et al.*, 1990; Ishida *et al.*, 1990b; Shinozaki & Ishida, 1991; Hayashi *et al.*, 1992;Krogsgaard-Larsen & Hansen, 1992; Schoepp *et al.*, 1992), possess a glutamate skeleton in their molecules which takes an extended form. Although the active form of other agonists for metabotropic glutamate receptors is not always known, it seems reasonable to assume that one of the possible conformations for activating metabotropic glutamate receptors is an extended form (Ishida *et al.*, 1990b). Both L-CCG-I and (1S,3R)-ACPD stimulate phosphoinositide hydrolysis, and L-CCG-I reduces the aden-

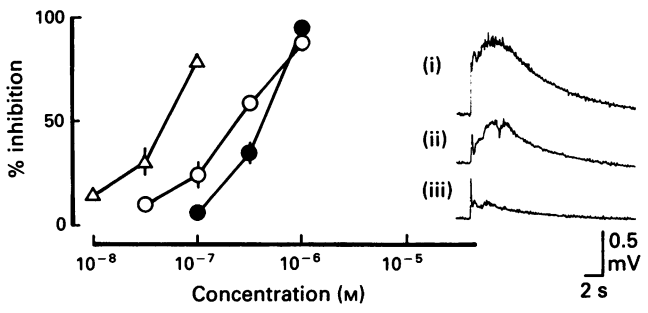

Figure 7 Reduction of the evoked reflex responses derived from L5 ventral roots, which were induced by an electrical stimulation (10 V, 300 μs duration, 180 s intervals) of the contralateral L5 dorsal root (contralateral slow DR-VRP) of the newborn rat (3 or 4-day-old). Left: Dose-response curves for baclofen (Δ), L-2-amino-4-phosphonobutanoic acid (L-AP4) (○) and (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) (●). Percentage inhibition of amplitudes of the contralateral slow DR-VRP was plotted against the concentrations. (i) Control, contralateral slow DR-VRP; (ii) DCG-IV (0.3 μM); (iii) baclofen (0.1 μM).

ylcyclase activity one order of magnitude more effectively than (1S,3R)-ACPD (Hayashi *et al.*, 1992). DCG-IV possesses the L-glutamate skeleton of an extended form which is quite similar to that of L-CCG-I, and DCG-IV does not stimulate phosphoinositide formation unlike L-CCG-I, but decreases forskolin-induced cyclic AMP formation as well as L-CCG-I (Maruyama *et al.*, unpublished observation). Therefore, DCG-IV is expected to be an agonist for metabotropic glutamate receptors, although there are some differences in the neuropharmacological actions of L-CCG-I and DCG-IV. The pharmacological profile of the recently cloned 'mGluR2' glutamate receptors which is negatively coupled to adenyl cyclase would be expected to be different from that of the metabotropic glutamate receptor coupled to phospholipase C. In the present study, DCG-IV did not cause any depolarization of motoneurones except for that mediated by NMDA receptors, while L-CCG-I and (1S,3R)-ACPD caused a depolarization that was to some extent decreased by 4C3H-PG; but both reduced preferentially the monosynaptic excitation of motoneurones at much lower concentrations than the threshold concentration for depolarization. The quisqualate-induced depolarization also consisted of various components including a metabotropic one, which was quite resistant to CPP and CNQX (Shinozaki *et al.*, 1989b). Quisqualate stimulated the cloned 'mGluR2' glutamate receptor expressed in Chinese hamster ovary cells less effectively than *trans*-ACPD (Nakanishi, 1992). Therefore, it seems probable that the depolarization caused by activating metabotropic glutamate receptors is a consequence of the stimulation of phosphoinositide hydrolysis in newborn rat spinal motoneurones.

At present, there are a few glutamate-related compounds such as DCG-IV that depress the monosynaptic excitation in the nanomolar range which is much lower than the threshold concentration for depolarization of motoneurones without affecting depolarizing responses to excitatory amino acids. Glutamic acid analogues which have been investigated in neural systems share with their parent compound two centres of negatively charged substituents and one with a positive charge. These structural features are believed to be essential for interaction of glutamic acid as an agonist with its neurotransmitter receptors. The structural homology of DCG-IV and L-CCG-I with glutamic acid is particularly close, and DCG-IV differs only by replacement of the hydrogen atom with a carboxylic group moiety on the C3' position of CCG, i.e. DCG-IV is one of the structural isomers of 3'-carboxylated CCG (see Figure 1). Attachment of the (3'R)-methoxymethyl and benzyloxymethyl group to the C3' position of L-CCG-IV (a potent NMDA agonist) converted the NMDA-like property into the non-NMDA type. (3'R)-methoxymethyl and benzyloxymethyl CCG derivatives caused kainate-like depolarization of the dorsal root fibre of newborn rats, while the (3'S)-CCG derivative demonstrated similar properties to parent compound, L-CCG-IV (Ishida *et al.*,1991). It is of great interest that the NMDA-like depolarizing activity of DCG-IV is less than that of L-CCG-IV, and, in addition, that the ability to stimulate phosphoinositide hydrolysis is abolished by 3'R-carboxylation. In passing, a tricarboxylic amino acid, L-4-carboxyglutamic acid (0.1 mM), reduced the monosynaptic component of ipsilateral DR-VRP in a similar manner to DCG-IV without reducing the polysynaptic discharges or causing postsynaptic depolarization, although the threshold concentration was markedly higher than that of DCG-IV (unpublished observation). This does not exclude the possibility that three centres of negatively charged substituents may be effective for activation of metabotropic glutamate receptors. In any case, C3'-substituted CCG compounds would provide useful information about the physiological function of excitatory amino acids.

The structural homology of DCG-IV with L-AP4 is not particularly close, but DCG-IV certainly stimulates L-AP4 receptors; a pharmacology based on antagonists for the L-AP4 receptor does not yet exist, and few known structural analogues of excitatory amino acids mimic the activity and potency of L-AP4, although recently some conformationally restricted cyclic analogues of L-AP4 have been synthesized (Koerner & Johnson, 1992). The L-AP4 receptor, like metabotropic glutamate receptors, operates via a second messenger system, but the most likely second messenger for the ON-bipolar cell L-AP4 receptor is cyclic GMP (Nawy & Jahr, 1990; Schiells & Falk, 1990). This fact, and the circumstance that known L-AP4 receptors and metabotropic glutamate receptors are pharmacologically and functionally distinct, justifies placing them in separate subclasses (Koerner & Johnson, 1992). In the present study, there were differences in the neuropharmacological actions of DCG-IV and L-AP4. DCG-IV completely reduced the DR-VRP monosynaptic component at the nanomolar range, but both monosynaptic excitation and polysynaptic discharges still occurred but at lower amplitudes in the presence of higher concentrations of L-AP4. Furthermore, in the case of the contralateral slow DR-VRP, L-AP4 attenuated it more effectively than DCG-IV, contrary to the case of the monosynaptic component of the ipsilateral DR-VRP, suggesting a difference in the distribution of their receptors.

It has been suggested that monosynaptic transmission of motoneurones may be mediated through non-NMDA receptors (Jahr & Yoshioka, 1986). Since DCG-IV did not affect the depolarizing responses to excitatory agonists for ionotropic glutamate receptors, the reduction of the DR-VRP monosynaptic component caused by DCG-IV would be expected to be a result of activation of presynaptic events, in addition, this is not due to activation of presynaptic ionotropic glutamate receptors, namely AMPA, kainate and NMDA receptors. Metabotropic glutamate receptors seem to regulate transmitter release in the hippocampus and other sites (Baskys & Malenka, 1991; Lovinger, 1991; Sunter *et al.*, 1991). In the present study, it is suggested that DCG-IV and L-CCG-I act on presynaptic terminals of primary afferent fibres directly or through activation of interneurones to decrease transmitter release, although their effective concentrations are considerably different. In the TTX-free ACSF, the baseline of the potential derived from the ventral root fluctuated considerably. The baseline fluctuation is due to spontaneous discharges through the release of neurotransmitters from nerve terminals. The fact that fluctuation of the baseline was abolished by L-CCG-I, L-AP4 and DCG-IV, could be explained by their block of transmitter release. On the other hand, (1S,3R)-ACPD did not reduce the fluctuation but rather enlarged it to increase excitability of motoneurones.

These results may differentiate their pharmacological properties to some extent.

The metabotropic glutamate receptors seem to be decreased in number or sensitivity due to developmental regulation with the lapse of days after birth (Nicolleti *et al.*,1986; Palmer *et al.*, 1990; Baskys & Malenka, 1991); therefore, the question remains as to whether the actions of DCG-IV and L-CCG-I are not common to those of the adult rats. However, when DCG-IV and L-CCG-I were given intraventricularly to the adult rat at a dose of 5–10 nmol or 50–600 nmol, respectively, both compounds caused marked sedation in rats, although there was a difference in the course of the development of sedation between L-CCG-I and DCG-IV. DCG-IV often caused a sedative effect after an excitatory stage in which the rat sometimes jumped abruptly or extended the hind limb convulsively. When 100 nmol of DCG-IV was administered intraventrically to the rat, the rat died from inhibition of respiration within about 10 min, while 600 nmol of L-CCG-I caused only marked reduction of muscle tone in the whole body, and this condition continued for more than 1 h. These results suggest that DCG-IV and L-CCG-I are still active even in adult rats, although receptors for DCG-IV and L-CCG-I may decrease in number or activity after birth. DCG-IV is expected to provide further useful information on the physiological function of metabotropic glutamate receptors.

This work was supported in part by Grant-in-Aids for Scientific Research from the Ministry of Education, Science and Culture of Japan to M.I. and H.S., and a Grant-in-Aid for Scientific Research on Priority Area from the Ministry of Education, Science and Culture of Japan to H.S.

References

AKAGI, H. & YANAGISAWA, M. (1987). GABAergic modulation of a substance P-mediated reflex of slow time course in the isolated rat spinal cord. *Br. J. Pharmacol.*, **91**, 189–197.

BASKYS, A. & MALENKA, R.C. (1991). Agonists at metabotropic glutamate receptors presynaptically inhibit EPSCs in neonatal rat hippocampus. *J. Physiol.*, **444**, 687–701.

BOSS, V., DESAI, M.A., SMITH, T.S. & CONN, P.J. (1992). Trans-ACPD-induced phosphoinositide hydrolysis and modulation of hippocampal pyramidal cell excitability do not undergo parallel developmental regulation. *Brain Res.*, **594**, 181–188.

DAVIES, J. & WATKINS, J.C. (1982). Actions of D- and L-forms of 2-amino-5-phosphonovalerate and 2-amino-4-phosphonobutyrate in the cat spinal cord. *Brain Res.*, **235**, 378–386.

DESAI, M.A. & CONN, P.J. (1991). Excitatory effects of ACPD receptor activation in the hippocampus are mediated by direct effects on pyramidal cells and blockade of synaptic inhibition. *J. Neurophysiol.*, **66**, 40–52.

EVANS, R.H., FRANCIS, A.A., JONES, A.W., SMITH, D.A.S. & WATKINS, J.C. (1982). The effects of a series of ω-phosphonic-α-carboxylic amino acids on electrically evoked and excitant amino acid-induced responses in isolated spinal cord preparations. *Br. J. Pharmacol.*, **75**, 65–75.

HAYASHI, Y., TANABE, Y., ARAMORI, I., MASU, M., SHIMAMOTO, K., OHFUNE, Y. & NAKANISHI, S. (1992). Agonist analysis of 2-(carboxycyclopropyl)glycine isomers for cloned metabotropic glutamate receptor subtypes expressed in chinese hamster ovary cells. *Br. J. Pharmacol.*, **107**, 539–543.

HILL, D.R. (1985). $GABA_B$ receptor modulation of adenylate cyclase activity in rat brain slices. *Br. J. Pharmacol.*, **84**, 249–257.

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., SHIMADA, Y. & SHINOZAKI, H. (1990a). A new potent metabotropic glutamate receptor agonist. *Soc. Neurosci.*, **16**, 1182P.

ISHIDA, M., AKAGI, H., SHIMAMOTO, K., OHFUNE, Y. & SHINOZAKI, H. (1990b). 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., OHFUNE, Y., SHIMADA, Y., SHIMAMOTO, K. & 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. (1984). Glutamate inhibitory action of matrine at the crayfish neuromuscular junction. *Br. J. Pharmacol.*, **82**, 523–531.

JAHR, C.E. & YOSHIOKA, K. (1986). Ia afferent excitation of motoneurones in the in vitro new-born rat spinal cord is selectively antagonized by kynurenate. *J. Physiol.*, **370**, 515–530.

JONES, P.L.St.J., PORTER, R.H.P., BIRSE, E.F., POOK, P.C.-K., SUNTER, D.C., UDVARHELYI, P.M., WHARTON, B., ROBERTS, P.J. & WATKINS, J.C. (1992). Characterization of a new excitatory amino acid (EAA) receptor type activated by (1S,3R)-ACPD and selectively blocked by (S)-4C3H-PG. *Br. J. Pharmacol.*, **103**, 47P.

KARBON, E.W. & ENNA, S.J. (1985). Characterization of the relationship between gamma-aminobutyric acid B agonists and transmitter-coupled cyclic nucleotide-generating systems in rat brain. *Mol. Pharmacol.*, **27**, 53–59.

KAWAI, M., HORIKAWA, Y., ISHIHARA, T., SHIMAMOTO, K. & OHFUNE, Y. (1992). 2-(Carboxycyclopropyl)glycines: binding, neurotoxicity and induction of intracellular free Ca^{2+} increase. *Eur. J. Pharmacol.*, **211**, 195–202.

KOERNER, J.F. & COTMAN, C.W. (1981). Micromolar L-2-amino-4-phosphonobutyric acid selectively inhibits perforant path synapses from lateral entorhinal cortex. *Brain Res.*, **216**, 192–198.

KOERNER, J.F. & JOHNSON, R.L. (1992). L-AP4 receptor ligands. In *Excitatory Amino Acid Receptors*, pp. 308–330. ed. Krogsgaard-Larsen, P. & Hansen, J.J. Chichester: Ellis Horwood.

KROGSGAARD-LARSEN, P. & HANSEN, J.J. (1992). *Excitatory Amino Acid Receptors*. Chichester: Ellis Horwood.

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.

LOVINGER, D.M. (1991). *Trans*-1-aminocylcopentane-1,3-dicarboxylic acid (t-ACPD) decreases synaptic excitation in rat striatal slices through a presynaptic action. *Neurosci. Lett.*, **129**, 17–21.

MILLER, R.J. (1991). Metabotropic excitatory amino acid receptors reveal their true colors. *Trends Pharmacol. Sci.*, **12**, 365–367.

MONAGHAN, D.T., BRIDGES, R.J. & COTMAN, C.W. (1989). The excitatory amino acid receptors: their 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.

NAKANISHI, S. (1992). Molecular diversity of glutamate receptors and implications for brain function. *Science*, **258**, 597–603.

NAWY, S. & JAHR, C.E. (1990). Suppression by glutamate of cGMP-activated conductance in retinal bipolar cells. *Nature*, **346**, 269–271.

NICOLETTI, F., IADAROLA, M.J., WROBLEWSKI, J.T. & COSTA, E. (1986). Excitatory amino acid recognition sites coupled with inositol phospholipid metabolism: developmental changes in interaction with α1-adrenoceptors. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 1931–1935.

OHFUNE, Y., SHIMAMOTO, K., ISHIDA, M. & SHINOZAKI, H. (1993). Synthesis of L-2-(2,3-dicarboxycyclopropyl)glycine: novel conformationally restricted glutamate analog. *Bioorg. & Med. Chem. Lett.*, **3**, 15–18.

PALMER, E., MONAGHAN, D.T. & COTMAN, C.W. (1989). *Trans*-ACPD, a selective agonist of the phosphoinositide-coupled excitatory amino acid receptor. *Eur. J. Pharmacol.*, **166**, 585–587.

PALMER, E., NANGEL-TAYLOR, K., KRAUSE, J., ROXAS, A. & COTMAN, C.W. (1990). Changes in excitatory amino acid modulation of phosphoinositide metabolism during development. *Develop. Brain Res.*, **51**, 132–134.

PORTER, R.H.P., ROBERTS, P.J., JANE, D.E. & WATKINS, J.C. (1992). (S)-homoquisqualate: a potent agonist at the glutamate metabotropic receptor. *Br. J. Pharmacol.*, **106,** 509–510.

SCHOEPP, D.D., BOCKAERT, J. & SLADECZEK, F. (1990). Pharmacological and functional characteristics of metabotropic excitatory amino acid receptors. *Trends Pharmacol. Sci.*, **11,** 508–515.

SCHOEPP, D.D., JOHNSON, B.G. & MONN, J.A. (1992). Inhibition of cyclic AMP formation by a selective metabotropic glutamate receptor agonist. *J. Neurochem.*, **58,** 1184–1186.

SHIELLS, R.A. & FALK, G. (1990). Glutamate receptors of rod bipolar cells are linked to a cyclic cascade via a G-protein. *Proc. R. Soc. B.*, **242,** 91–94.

SHINOZAKI, H. & ISHIDA, M. (1978). Theanine as a glutamate antagonist at a crayfish neuromuscular junction. *Brain Res.*, **151,** 215–219.

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 neurones and crayfish neuromuscular junction. *Comp. Biochem. Physiol.*, **103C,** 13–17.

SHINOZAKI, H. & ISHIDA, M. (1993). Excitatory amino acids: physiological and pharmacological probes for neuroscience research. *Acta Neurobiol. Exp.*, **53,** 43–51.

SHINOZAKI, H., ISHIDA, M. & KUDO, Y. (1992). Potent NMDA like actions of conformational variants of a glutamate analogue. In *NMDA Receptor Related Agents*, pp. 247–260. ed. Kameyama, T., Nabeshima, T. & Domino, E.F. Ann Arbor: NPP Books.

SHINOZAKI, H., ISHIDA, M., SHIMAMOTO, K. & OHFUNE, Y. (1989a). 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. & OHFUNE, Y. (1989b). Potent NMDA-like actions and marked potentiation of glutamate responses of conformational variants of a glutamate analogue in the rat spinal cord. *Br. J. Pharmacol.*, **98,** 1213–1224.

SIMON, R.P. (1992). *Excitatory Amino Acids*, (ed.) FIDIA Research Foundation Symposium Series, Vol. 9. New York, Thieme.

SLADECZEK, F., PIN, J.P., RECASENS, M., BOCKAERT, J. & WEISS, S. (1985). Glutamate stimulates inositol phosphate formation in striatal neurons. *Nature*, **317,** 717–719.

SUGIYAMA, H., ITO, I. & HIRONO, C. (1987). A new type of glutamate receptor linked to inositol phospholipid metabolism. *Nature*, **325,** 531–533.

SUGIYAMA, H., ITO, I. & WATANABE, M. (1989). Glutamate receptor subtypes may be classified into two major categories: a study on Xenopus oocytes injected with rat brain mRNA. *Neuron*, **3,** 129–132.

SUNTER, D.C., EDGAR, G.E., POOK, P.C.-K., HOWARD, J.A.K., UDVARHELYI, P.M. & WATKINS, J.C. (1991). Actions of the four isomers of 1-aminocyclopentane-1,3-dicarboxylate (ACPD) in the hemisected isolated spinal cord of the neonatal rat. *Br. J. Pharmacol.*, **104,** 377P.

TANABE, Y., MASU, M., ISHII, T., SHIGEMOTO, R. & NAKANISHI, S. (1992). A family of metabotropic glutamate receptors. *Neuron*, **8,** 169–179.

YAMAMOTO, C., SAWADA, S. & TAKADA, S. (1983). Suppressing action of 2-amino-4-phosphonobutyric acid on mossy fiber-induced excitation in the guinea pig hippocampus. *Exp. Brain Res.*, **51,** 128–134.

(Received January 18, 1993
Revised March 8, 1993
Accepted March 30, 1993)