Evidence for Endogenous Excitatory Amino Acids as Mediators in DSI of GABA_\text{A}ergic Transmission in Hippocampal CA1

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Morishita, Wade and Bradley E. Alger. Evidence for endogenous excitatory amino acids as mediators in DSI of GABA_\text{A}ergic transmission in hippocampal CA1. J. Neurophysiol. 82: 2556–2564, 1999. Depolarization-induced suppression of inhibition (DSI) is a process whereby brief (∼ 1-s) depolarization to the postsynaptic membrane of hippocampal CA1 pyramidal cells results in a transient suppression of GABA_\text{A}ergic synaptic transmission. DSI is triggered by a postsynaptic rise in [Ca^{2+}]_i, and yet is expressed presynaptically, which implies that a retrograde signal is involved. Recent evidence based on synthetic metabotropic glutamate receptor (mGluR) agonists and antagonists suggested that group I mGluRs take part in the expression of DSI and raised the possibility that glutamate or a glutamate-like substance is the retrograde messenger in hippocampal CA1. This hypothesis was tested, and it was found that the endogenous amino acids L-glutamate (L-Glu) and L-cysteine sulfonic acid (L-CSA) suppressed GABA_\text{A}-receptor–mediated inhibitory postsynaptic currents (IPSCs) and occluded DSI, whereas L-homocysteic acid (L-HCA) and L-homocysteine sulfinic acid (L-HCSA) did not. Activation of metabotropic kainate receptors with kainic acid (KA) reduced IPSCs; however, DSI was not occluded. When iontophoresitically applied, both L-Glu and L-CSA produced a transient IPSC suppression similar in magnitude and time course to that observed during DSI. Both DSI and the actions of the amino acids were antagonized by (S)-a-methyl-D-carboxyphenylglycine ([S]-MCPG), indicating that the effects of the endogenous agonists were produced through activation of mGluRs. Blocking excitatory amino acid transport significantly increased DSI and the suppression produced by L-Glu or L-CSA without affecting the time constant of recovery from the suppression. Similar to DSI, IPSC suppression by L-Glu or L-CSA was blocked by N-ethylmaleimide (NEM). Moreover, paired-pulse depression (PPD), which is unaltered during DSI, is also not significantly affected by the amino acids. Taken together, these results support the glutamate hypothesis of DSI and argue that L-Glu or L-CSA are potential retrograde messengers in CA1.

INTRODUCTION

In hippocampal CA1 pyramidal cells (Alger et al. 1996; Pitler and Alger 1992, 1994) and in cerebellar Purkinje cells (Llano et al. 1991; Vincent et al. 1992; Vincent and Marty 1993), brief (∼ 1-s) influx of Ca^{2+} through voltage-dependent Ca^{2+} channels produces a transient suppression of GABA_\text{A}ergic inhibitory postsynaptic currents (IPSCs). This phenomenon, termed depolarization-induced suppression of inhibition (DSI), appears to be mediated by a retrograde message released after depolarization of the pyramidal cell. This hypothesis is based on a great deal of recent work. DSI is not accompanied by detectable changes in GABA_\text{A} receptor response properties, whether these are assessed by iontophoretic GABA responses (Llano et al. 1991; Pitler and Alger 1992) or by spontaneous (Llano et al. 1991; Pitler and Alger 1994), evoked (Alger et al. 1996), or Sr^{2+}-induced (Morishita and Alger 1997) miniature IPSCs. DSI is associated with a decrease in GABA release as measured by the coefficient of variation (Alger et al. 1996), the method of failures (Alger et al. 1996; Vincent et al. 1992), or direct counting of evoked asynchronous quanta in Sr^{2+} (Morishita and Alger 1997). Evidently, Ca^{2+} influx into a pyramidal cell causes a signal to be sent to interneurons that prevents them from releasing GABA efficiently for a time.

Although the identity of this signal is not fully determined, recent experiments on the cerebellum (Glitsch et al. 1996) and hippocampus (Morishita et al. 1998) have implicated activation of metabotropic glutamate receptors (mGluRs) in the DSI process. The mGluR family consists of eight subtypes of G-protein–coupled receptors: group I (mGluRs 1 and 5), group II (mGluRs 2 and 3), and group III (mGluRs 4, 6, 7, 8), which can be distinguished by pharmacological and biochemical criteria (Pin and Duvoisin 1995). Support for the metabotropic glutamate hypothesis of DSI includes the observations based on the use of synthetic mGluR agonists. In the cerebellum, the group II agonist DCG-IV mimics and occludes DSI (Glitsch et al. 1996), whereas in hippocampal CA1 the selective group I mGluR agonists quisqualate (at a low concentration) and DHPG have the same effects (Morishita et al. 1998), and participation of group II or III receptors can be ruled out. In CA1, the group I and group II antagonist (S)-α-methyl-D-carboxyphenylglycine (S-MCPG) reduced both the synthetic agonist effects and DSI.

These findings support the hypothesis that glutamate or a glutamate-like substance could be the retrograde signal in DSI. Nevertheless, the mGluR-glutamate hypothesis has not been tested using endogenous amino acids in any preparation, and, except for the block of DSI by MCPG in hippocampus, the hypothesis is based mainly on similarity of action between synthetic mGluR agonists and DSI. We examined the actions of L-glutamate (L-Glu) and several endogenous amino acids on IPSCs and DSI. Although a number of neurotransmitter candidates exist in the CNS, we focused on the sulfur-containing amino acids, L-homocysteic acid (L-HCA), L-homocysteine sulfinic acid (L-HCSA), and L-cysteine sulfinic acid (L-CSA), because they can activate group I mGluRs (Kingston et al. 1998). We also examined the possibility of a role for the metabotropic kainic acid (KA) receptor (Rodriguez-Moreno and Lerman 1998) in DSI. This KA receptor is present on interneurons and, when activated, produces a G-protein–mediated presynaptic suppression of IPSCs onto CA1 pyramidal neurons.
cells (Clarke et al. 1997; Cossart et al. 1998; Fisher and Alger 1984; Freking et al. 1998; Rodriguez-Moreno et al. 1997). The crucial prediction of the mGluR-glutamate hypothesis is that endogenous excitatory amino acid agonists of mGluRs should meet criteria established for the DSI messenger. The data indicate that L-Glu or L-CSA are good candidates for the retrograde messenger in DSI in CA1, but that L-HCA and L-HCSA are not. A role for KA receptors in DSI can be ruled out. Some of the results in this study have appeared in abstract form (Morishita and Alger 1998).

METHODS

Preparation of slices

Transversely sectioned slices 400-μm thick were obtained from the hippocampi of adult male Sprague-Dawley rats (125–250 g) as previously described (Morishita and Alger 1997). The slices were maintained at room temperature in a holding chamber at the interface of a physiological saline and humidified 95% -5% CO2 mixture. After a minimum of 1 h of incubation, a single slice was transferred to a submersion-type recording chamber (Nicoll and Alger 1981) where it was perfused with saline (29–31°C) at a flow rate of 0.5–1 ml/min.

Solutions

The physiological saline comprised (in mM): 120 NaCl, 3 KCl, 1 NaH2PO4, 25 NaHCO3, 2.5 CaCl2, 2 MgSO4, and 10 glucose. In all experiments, 6-cyano-7-nitroquinoline-2,3-dione (CNQX, 40 or 100 μM) and either (S)-10-11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK-801, 100 μM) or d.L-2-amino-5-phosphonovaleric acid (APV, 100 μM) was added to the physiological saline to block ionotropic glutamate-receptor–mediated responses. The noncompetitive α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist, 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine hydrochloride (GYKI 52466, 50 μM) was positioned in stratum pyramidale (Morishita and Alger, 1997). The slices were maintained at room temperature in a holding chamber at the interface of a physiological saline and humidified 95% -5% CO2 mixture. After a minimum of 1 h of incubation, a single slice was transferred to a submersion-type recording chamber (Nicoll and Alger 1981) where it was perfused with saline (29–31°C) at a flow rate of 0.5–1 ml/min.

Whole cell voltage-clamp recordings were made from CA1 pyramidal cells. The cells were clamped at −70 mV, and IPSCs were evoked by depolarizing the postsynaptic membrane to 0 or −10 mV for durations ranging between 0.5 and 3 s every 90 s. Iontophoretic pipettes, filled with L-Glu (100 mM or 1 M, pH 9) or L-CSA (50 or 100 mM, pH 9), were positioned in stratum pyramidale in proximity (50–100 μm) to the recording electrode. A 20-nA retaining current was applied to the iontophoretic pipettes to minimize leakage of drugs, and iontophoretic applications (performed with ejecting currents of 150–170 nA with durations ranging between 0.5 and 4 s) were alternated with the DSI-inducing voltage step.

Current signals were filtered at 2 kHz with an eight-pole Bessel filter (Frequency Devices, Haverhill, MA), digitized at 10 kHz (DigiData 1200, Axon Instruments, Foster City, CA), and stored on a pentium-based personal computer (Dell Dimensions XPS M200). The signals were also acquired at 22 kHz with a 14-bit PCM digitizer (Neuro-Corder DR-484, Neuro Data Instruments) and stored on VHS videotape.

Data analysis

To quantify peak DSI the following formula was used

\[ DSI = \left(1 - \frac{IPSC}{IPSC_c}\right) \times 100\% \tag{1} \]

where \( IPSC \) is the mean amplitude of seven consecutive IPSCs evoked during the control period (the period immediately before the DSI-inducing voltage step), and \( IPSC_c \) is the mean of five consecutive IPSCs elicited during the test period (the period after the voltage step). The first IPSC after the voltage step was not used in calculating \( IPSC_c \), because it takes 1–2 s for DSI to develop (Pitler and Alger 1994). This formula allowed an accurate measurement of DSI and was used to quantify the effects of drugs on DSI.

Nonlinear regression analysis was used to measure the time constant of recovery from DSI. The time course of recovery was best fitted by the single exponential equation

\[ y = y_o + y_{\text{min}} \times e^{-t/\tau} \tag{2} \]

where \( y \) is the normalized magnitude of the IPSC at time \( t \), \( y_o \) is an adjustable fitting parameter, \( y_{\text{min}} \) is the normalized magnitude of the IPSC at the peak of DSI, and \( \tau \) is the time constant of the DSI process.

Off-line analysis was performed using pClamp 6 (Axon Instruments, Foster City, CA) and SigmaPlot 4 (SPSS, Chicago, IL) software. Statistical analysis of the data were performed using a Student’s paired t-test (\( P < 0.05 \)).

RESULTS

We began by determining whether bath application of 1–5 mM L-Glu and L-CSA could mimic or occlude DSI. Typical experiments are shown in Fig. 1. A1 and B1. The dots represent the peak amplitudes of individual monosynaptic IPSCs that were evoked at 3-s intervals. DSI was elicited by a depolarizing voltage step at 90-s intervals (given at the points marked by the arrows). After each voltage step, the IPSC amplitudes were reduced and then recovered to control levels over the next minute. Note that both agents, when bath applied for ~7 min, strongly and reversibly suppressed the IPSCs (IPSC reduction in L-Glu, 53 ± 9.2%; in L-CSA, 69 ± 7.7%; Fig. 1, A3, n = 4 and B3, n = 5) and occluded DSI. DSI was 61 ± 5.6% in control and 36 ± 5.2% during L-CSA and 46 ± 7.9% before and 20 ± 7.1% during L-Glu application (Fig. 1, A3, n = 4 and B3, n = 5, respectively). After washout of L-Glu or L-CSA, the IPSCs and DSI recovered to near control values. If, when DSI was reduced, the stimulation intensity was increased to elicit IPSCs comparable in amplitude with those in control (in L-Glu, n = 2, Fig. 1A2; in L-CSA n = 3, Fig. 1B2),
DSI remained reduced, indicating that the actions of these agonists were on the DSI process and could not be accounted for by a simple reduction in IPSC size.

Bath application of two other sulfur-containing amino acids that are agonists at mGluRs (Kingston et al. 1998), 1 mM L-HCA and 1–1.5 mM L-HCSA, produced effects unlike those of L-Glu or L-CSA, 1-HCA reduced IPSCs by 95.6 ± 7.8%, but did not occlude DSI, which was 67 ± 8.5% in control and 65 ± 2.9% in L-HCA (n = 3). L-HCSA also reduced IPSCs by 88 ± 9.9% without occluding DSI. L-HCSA actually increased DSI as calculated from Eq. 1, from 37 ± 6.8% to 65 ± 3.1% (n = 3), although whether this represents an enhancement of the DSI process or some other factor cannot be determined from the present data. In any case, their failure to occlude DSI argues that neither L-HCA nor L-HCSA is a strong candidate as the DSI messenger, and we did not consider them further.

As previously reported (Cossart et al. 1998; Fisher and Alger 1984; Frerking et al. 1998; Rodriguez-Moreno and Lerma 1998; Rodriguez-Moreno et al. 1997), KA (10 μM) also suppressed IPSCs (IPSC reduction in KA, 49 ± 4.5%; Fig. 1C2, n = 5); however, unlike L-Glu and L-CSA, KA did not occlude DSI (control DSI, 45 ± 3.4%; KA DSI, 46 ± 3.7%, n = 5, Fig. 1C3).
Additional evidence that activation of KA receptors does not play a role in DSI can be seen in the effects of subsequent application of 100 μM CNQX. At 100 μM, CNQX reversed the KA-induced suppression of IPSCs. Concomitantly, as the IPSC amplitudes returned to near control values, DSI also became more readily detectable (KA + CNQX DSI, 51 ± 3.9%). Had the KA receptor been involved in DSI, the IPSCs would have recovered, and DSI would have been blocked. The recovery of IPSCs and the presence of DSI in 100 μM CNQX can be contrasted with the effects of the mGluR antagonist MCPG, which reverses the effects of (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid [(1S,3R)-ACPD] on IPSC amplitudes, and yet simultaneously reduces DSI (Fig. 3, cf. Morishita et al. 1998).

The occlusion experiments shown in Fig. 1 suggested that L-Glu or L-CSA could be candidates for the DSI signal. We tested this hypothesis in more detail using iontophoresis. Brief application of a candidate messenger (rather than the bath application shown in Fig. 1) may be expected to mimic the time course of the endogenous agent. In the case of a fast, ionotopic neurotransmitter response, very rapid application of the suspected transmitter very close to the receptors is required (e.g., Kuffler and Yoshikami 1975; Liu et al. 1999). However, the requirements for speed and precise location of application are less critical in the case of an mGluR-mediated action. Brief DCG-IV application to cerebellar cells produced IPSC suppression with a time course similar to that of cerebellar DSI (Glitsch et al. 1996). The mGluRs are often located in perisynaptic regions outside the immediate subsynaptic zone (Lujan et al. 1996) and therefore are probably not subject to the same rapid exposure to synaptically released glutamate. In addition, the biochemical cascades downstream of activation of metabotropic receptors should have a greater influence on the overall duration of the response than on the receptor-binding steps. We therefore iontophoresed L-Glu or L-CSA to CA1 cells while continuously evoking monosynaptic IPSCs. To compare the agonist effects and DSI, we alternated iontophoretic applications with DSI trials at 90-s intervals. Iontophoretic applications of L-Glu (Fig. 2A) and L-CSA (Fig. 2B) produced transient reductions in IPSCs. Similar maximal peak IPSC reductions induced with DSI or amino acid iontophoresis recovered with very similar time courses, as shown in the examples at the top and the group data in the bottom graphs (L-Glu, n = 10; L-CSA, n = 7).
By alternating DSI trials with iontophoretic application of either L-Glu or L-CSA, we found that the effects of both agonists and DSI can be antagonized by (S)-MCPG (cf. Morishita et al. 1998). (S)-MCPG (2.5–3 mM) caused a significant, and comparable, decrease in the IPSC-suppressive effects of both agonists and DSI. Control DSI and L-Glu reduction of IPSCs were 54 ± 6.4% and 47 ± 3.1%, respectively. In (S)-MCPG, DSI and L-Glu suppression of IPSCs were 33 ± 6.3% and 31 ± 1.9%, respectively (Fig. 3A, n = 6). Control DSI and L-CSA reduction of IPSCs were 54 ± 7.1% and 24 ± 6.7%, respectively (Fig. 3B, n = 4). The data confirm that the agonists reduce IPSCs by acting on mGluRs.

If a glutamate-like agonist is the DSI signal, then DSI should be affected by inhibition of glutamate uptake systems. Both L-Glu and L-CSA are substrates for glutamate transporters, and we therefore examined the effects of a combination of glutamate uptake inhibitors, DHK (1 mM) and THA (0.5 mM), on these parameters, by means of alternating iontophoretic applications and DSI induction. In these experiments the durations of DSI-inducing voltage steps and iontophoretic applications were either 1 or 2 s. The time constant of decay of DSI is independent of voltage-step duration (over a nearly 10-fold range of durations), provided a comparable degree of DSI is induced (Lenz and Alger 1999); therefore, data from these experiments can be compared. Figure 4 shows the results of these experiments. To maximize the chances of observing an effect of the uptake inhibitors, in these experiments we adjusted conditions to obtain minimal levels of IPSC suppression, by iontophoresis or DSI. Control values of DSI and of IPSC suppression by L-Glu or L-CSA (20–30%, rather than the 40–50% that are typical) were similar. In the presence of uptake inhibitors, DSI and IPSC suppression by L-Glu increased from 26 ± 8.8% to 39 ± 6.9% and from 20 ± 8.1% to 46 ± 10%, respectively (Fig. 4A, n = 5). DSI and IPSC suppression by L-CSA was also enhanced from 22 ± 5.3% to 37 ± 5.6% and from 25 ± 3.9% to 40 ± 3.7% (Fig. 4B, n = 7). These increases were significant; P < 0.05. We also examined the effects of the uptake inhibitors on the time constant of

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**Fig. 3.** (S)-α-methyl-4-carboxyphenylglycine (MCPG) antagonizes both DSI and the IPSC suppression induced by iontophoretic application of L-Glu or L-CSA. A: IPSCs during iontophoresis (open arrows) of L-Glu and during a voltage step (filled arrows) in control, in (S)-MCPG and after a recovery from (S)-MCPG. All current traces were recorded from the same cell. **Bottom, left:** averages of 5 consecutive IPSCs recorded at the indicated time points. **Bar graph summarizes results from 6 experiments.** B: same protocol as in A, but results were obtained with iontophoresis of L-CSA (n = 4 experiments). *Significant differences from control values (P < 0.05).
IPSC suppression by DSI and by the amino acids. The graph in Fig. 4 shows averaged data for the time courses of IPSC suppression and recovery in control and in the presence of the glutamate transporter blockers. Although blocking glutamate uptake produced greater IPSC suppression, it did not alter the time constant of recovery itself. The total duration of DSI was, naturally, enhanced because DSI was greater at every time point, as can be seen from the dotted lines in the graphs of Fig. 4. DHK and THA slowed the time course of fast ionotropic responses (cf. Tong and Jahr 1994, Fig. 4C, inset), indicating that they blocked glutamate transporters.

DSI has a number of properties that can be used to screen candidate DSI messengers. For instance, DSI and the (1S,3R)-ACPD–induced suppression of IPSCs are blocked by 250 μM NEM, before NEM markedly suppresses the IPSCs themselves (Morishita et al. 1997). We now show (Fig. 5) that NEM also blocked the effects of L-Glu and L-CSA on IPSCs (IPSC reduction by L-Glu and L-CSA, 54 ± 9.4% and 43 ± 3.1%, respectively; IPSC reduction by L-Glu and L-CSA in NEM, 9 ± 3.8% and 4 ± 2.8%, respectively, Fig. 5, A and B, n = 3).

An interesting property of DSI is that, although it represents a presynaptic inhibitory process, it is unlike many forms of conventional presynaptic inhibition in that it does not alter paired-pulse responsiveness (Alger et al. 1996; Morishita et al. 1998). Paired-pulse depression (PPD) is a prominent feature of monosynaptic IPSCs in the hippocampus (Davies et al. 1990). The second response to a pair of stimuli administered 200 ms after the first is typically suppressed relative to the first response, a reduction commonly expressed by the paired-pulse ratio as the amplitude of IPSC2 divided by that of IPSC1. DSI reduced IPSC amplitudes without altering PPD; both responses were decreased in parallel. L-Glu and L-CSA also reduced IPSCs without significantly altering the paired-pulse ratio. In control solution the PPD ratio was 0.83 ± 0.01; in L-Glu it was 0.81 ± 0.01, Fig. 6, A1 and A2, n = 5; in control solution the PPD ratio was 0.84 ± 0.01; and in L-CSA it was 0.86 ± 1.9 (Fig. 6, B1 and B2, n = 7).

DISCUSSION

Although mGluRs have been implicated in DSI in both cerebellum and hippocampus, endogenous amino acids have not been tested. Our data indicate that both L-Glu and another excitatory amino acid neurotransmitter candidate, L-CSA, meet...
criteria that should distinguish the DSI messenger. They mimicked and occluded DSI, their effects were blocked by NEM and (S)-MCPG, and they did not alter PPD. Other very similar amino acids, L-HCA and L-HCSA, did not occlude DSI. KA serves as a very important control in this case because, although it is a glutamate agonist and antagonizes evoked IPSCs (Clarke et al. 1997; Cossart et al. 1998; Fisher and Alger 1984; Frerking et al. 1998; Rodriguez-Moreno et al. 1997) at a presynaptic site linked to a G-protein (Rodriguez-Moreno and Lerma 1998), a role for the KA receptor in DSI could be ruled out. It was possible to reverse the effects of KA on IPSCs without altering DSI. Conversely, the mGluR antagonist (S)-MCPG antagonized the effects of L-Glu and L-CSA on IPSCs, as well as DSI, consistent with a role for mGluR agonists in DSI. At this point we cannot distinguish between L-Glu and L-CSA, and both must be considered equally good candidate retrograde messengers for DSI.

Glutamate is by far the best established excitatory amino acid neurotransmitter; however, other endogenous amino acid candidates exist. L-CSA is an especially interesting one that fulfills most of the commonly accepted criteria for transmitter identification, including Ca²⁺-dependent release. Besides acting on ionotropic (Conn et al. 1994) and metabotropic (Boss and Boaten 1995) glutamate receptors, L-CSA also acts on an excitatory amino acid receptor that is unique in catalyzing breakdown of membrane phospholipids through the activation of phospholipase D (PLD) (Boss et al. 1994). The PLD-coupled receptor is probably not involved in DSI because it is insensitive to glutamate and MCPG, whereas it is activated by L-AP3 (Conn et al. 1994). As shown in the present report, DSI is very effectively mimicked by glutamate, is blocked by MCPG and, as previously noted, is unaffected by L-AP3 (Morishita et al. 1998).

The broad-spectrum mGluR antagonist (S)-MCPG is more potent on mGluR1 than on mGluR5, and, even at mGluR5, is much more potent when (1S,3R)-ACPD is the agonist than when L-Glu is (Brabet et al. 1995; Joly et al. 1995; Littman and Robinson 1994). L-CSA, similar to L-Glu, is a full agonist in eliciting phosphoinositide turnover through activation of the group I human mGluR1α and mGluR5α when these receptors are expressed in the Syrian hamster tumor cell line AV12-664 (Kingston et al. 1998). Actions at these receptors are likely to be relevant for DSI. Synthetic agonists at group I receptors—(1S,3R)-ACPD, quisqualate at a low concentration and (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-I; high concentration)—mimic and occlude DSI, whereas the group II agonists DCG-IV and L-CCG-I (low concentration) have no effect on IPSCs in CA1 (Morishita et al. 1998; cf. Gereau and Conn 1995). Of the group I receptors, mGluR5 is the most likely candidate for mediating DSI in CA1, because mGluR1 receptors are confined to a subgroup of interneurons in s. oriens, whereas mGluR5s are densely distributed throughout CA1 (Shigemoto et al. 1997). The mGluR antagonist (S)-4-carboxyphenylglycine (4CPG), much more potent in blocking mGluR1α than mGluR5α (Brabet et al. 1995; Joly et al. 1995), had no effect on DSI or the reduction in IPSCs caused by

![Image](http://jn.physiology.org/doi/10.1152/jn.01089.2016)
1S,3R-ACPD (Morishita et al. 1998). Blocking the effects of \( \text{L-Glu} \) and \( \text{L-CSA} \) required high concentrations of MCPG, as did the antagonism of DSI. Thus, these data continue to point to an endogenous glutamate-like amino acid and mGluR5 as mediators of DSI.

The glutamate transport blockers DHK and THA enhanced DSI and the effects of \( \text{L-Glu} \) and \( \text{L-CSA} \). This adds to the number of close similarities between the actions of mGluR agonists and DSI. The specificity of DHK and THA provides a direct link between glutamate agonists and DSI. Although it is taken up by the glutamate transporter, \( \text{L-CSA} \) is not an ideal substrate and in fact can reduce glutamate uptake, giving rise to concerns that some of its effects could be caused by competitive heteroexchange (Kingston et al. 1998) for glutamate. If this were to explain the actions of \( \text{L-CSA} \) in our experiments it would strengthen the candidacy of glutamate as the retrograde signal in DSI. Although it is difficult to rule out this possibility completely, we think that it is not likely to be responsible for the effects of \( \text{L-CSA} \) in our experiments. The suppressive effect of \( \text{L-CSA} \) on IPSCs should have been occluded in the presence of the uptake blockers if it had been secondary to block of glutamate uptake. On the contrary, \( \text{L-CSA} \) produced a greater effect in the presence of DHK and THA.

The absence of effect of uptake inhibitors on the time constant of IPSC suppression and DSI is consistent with the interpretation that the availability of the ligand at the receptor is not limiting for the duration of metabotropic transmitter actions, which are largely functions of downstream effector steps. Inhibiting glutamate transporters may be equivalent to increasing the concentration of the ligand (Fitzsimonds and Dichter 1996), which could explain the enhancement of IPSC suppression by DSI and the mGluR agonists.

If \( \text{L-Glu} \) or \( \text{L-CSA} \) is the retrograde messenger in DSI, then an important challenge will be to elucidate its mechanism of release from the pyramidal cells. DSI is caused by \( \text{Ca}^{2+} \) influx into the somatodendritic regions of these cells, and yet no presynaptic specializations or concentrations of synaptic vesicles have been found in these regions. A recent study has shown that DSI has a \( \text{Ca}^{2+} \) dependence that in many ways more closely resembles that of hormonal, or neuropeptide, slow release than it does fast neurotransmitter release (Lenz and Alger 1999). It will be interesting to determine whether DSI involves a conventional release process.

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