ELSEVIER

European Journal of Pharmacology 307 (1996) 219–225

ejp

The metabotropic glutamate receptor antagonist L-2-amino-3-phosphonopropionic acid inhibits phosphoserine phosphatase

Jon E. Hawkinson *, Manuel Acosta-Burruel, Paul L. Wood

CoCensys, Inc., 213 Technology Drive, Irvine, CA 92718, USA

Received 18 January 1996; revised 6 March 1996; accepted 19 March 1996

Abstract

Phosphoserine phosphatase catalyzes the final step in the major pathway of L-serine biosynthesis in brain. Using D-phosphoserine as substrate, the metabotropic glutamate receptor antagonist L-2-amino-3-phosphonopropionic acid (L-AP3) inhibits phosphoserine phosphatase partially purified from rat brain with a K_i of 151 μM. In contrast to AP3 enantioselectivity at metabotropic receptors, D-AP3 (K_i 48 μM) is more potent as an inhibitor of phosphoserine phosphatase than L-AP3, whereas DL-AP3 has intermediate potency. D-, L-, and DL-AP3 are 6- to 8-fold more potent inhibitors using D-phosphoserine rather than L-phosphoserine as substrate, suggesting that AP3 may have selectivity for isoforms of phosphoserine phosphatase which preferentially cleave D-phosphoserine. D-AP3 decreases the apparent affinity of D- and L-phosphoserine with little or no change in maximal velocity indicating that it is a competitive inhibitor of the enzyme. Whereas L-AP3 has similar potency at metabotropic glutamate receptors and phosphoserine phosphatase, D-AP3 is selective for phosphoserine phosphatase and is the most potent and only known competitive inhibitor of this enzyme.

Keywords: Phosphoserine phosphatase; L-AP3 (L-2-amino-3-phosphonopropionic acid); D-AP3 (D-2-amino-3-phosphonopropionic acid); Serine; Glycine

1. Introduction

Phosphoserine phosphatase (EC 3.1.3.3) is a key enzyme in the biosynthetic pathway of L-serine. Phosphoserine phosphatase catalyzes the hydrolysis of L-phosphoserine to form L-serine and inorganic phosphate in the final step of the phosphorylated pathway of L-serine biosynthesis (Fig. 1). Although the alternate nonphosphorylated pathway exists in many tissues, greater than 90% of L-serine in brain is formed via the phosphorylated pathway (Bridgers, 1965). L-Serine regulates phosphoserine phosphatase activity via negative feedback inhibition that has been characterized as noncompetitive (Bridgers, 1967) or uncompetitive (Veeranna and Shetty, 1991) in nature. In addition to phosphatase activity, the enzyme also has phosphotransferase activity since it can transfer a phosphoryl group from L-phosphoserine to L-serine. These reactions apparently occur through different mechanisms since the activities are separable (Bridgers, 1967).

Phosphoserine phosphatase is a Mg^{2+}-dependent enzyme having an estimated molecular weight of 47–65 kDa (Bridgers, 1969; Moro-Furlani et al., 1980; Paoli et al., 1974), although this may represent a dimeric form of the enzyme with a monomeric molecular weight of 26 kDa (Moro-Furlani et al., 1980). Phosphoserine phosphatase self-associates to produce higher molecular weight forms via intermolecular disulfide bonds under certain conditions (Paoli et al., 1974). Multiple isozymes of human phosphoserine phosphatase from several tissues have been identified. Common isozymes probably arise from posttranslational modification, whereas rare forms are apparently due to allelic variation at an autosomal locus (Moro-Fur-

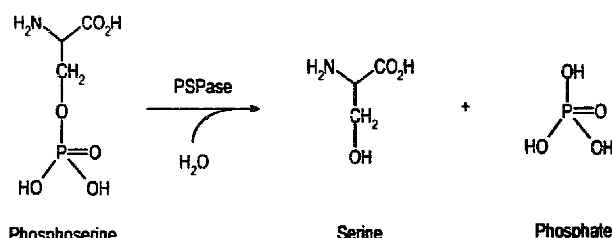

Fig. 1. Reaction catalyzed by phosphoserine phosphatase (PSPase).

* Corresponding author. Tel.: (714) 753-6100 ext. 243; fax: (714) 753-6161.

0014-2999/96/$15.00 Copyright © 1996 Elsevier Science B.V. All rights reserved.
PII S0014-2999(96)00253-1

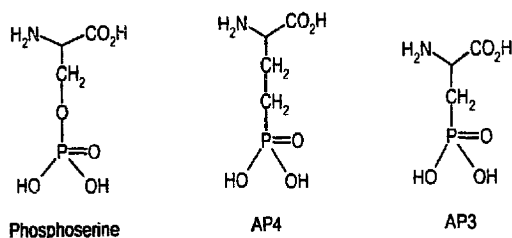

Fig. 2. Structures of phosphoserine, AP4, and AP3.

lani et al., 1980). The *serB* gene thought to encode phosphoserine phosphatase has been cloned from *E. coli* and predicts a protein of 322 amino acids with a molecular weight of 35 kDa (Neuwald and Stauffer, 1980).

Excitatory amino acid receptors are classified as ionotropic or metabotropic depending on whether they form ion channels or are coupled to G-proteins, respectively. Knowledge of the diversity of metabotropic receptors has outpaced their pharmacological characterization with the cloning of several subtypes ($mGlu_{1-8}$) coupled to phospholipase C or adenyl cyclase (Knopfel et al., 1995). Expression studies have clarified the subtype selectivity of the metabotropic agonists (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD), L-2-amino-4-phosphonobutyric acid (L-AP4), and (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine (L-CCG-I). Recent progress has been made in the development of antagonists such as *S*-4-carboxyphenylglycine (*S*-4CPG), which is a competitive antagonist at the $mGlu_{1\alpha}$ and $mGlu_2$ receptor subtypes, and α-methyl-L-AP4 (MAP4) and α-methyl-L-CCG-I (MCCG), whose subtype selectivity is unknown (Knopfel et al., 1995).

Racemic 2-amino-3-phosphonopropionic acid (DL-AP3) (Fig. 2) was identified as a metabotropic receptor antagonist by its inhibition of ibotenate-stimulated phosphoinositide hydrolysis in rat hippocampal slices (Schoepp and Johnson, 1989). This activity was later shown to reside primarily in the L-isomer (Schoepp and Conn, 1993). However, the actions of L-AP3 are complex since it has been reported to have antagonist or partial agonist activity for phosphoinositide hydrolysis in rat hippocampal slices but is relatively inactive in cerebellar slices (Schoepp and Conn, 1993). L-AP3 also antagonizes long-term potentiation induced by L-AP4 presumably acting via metabotropic receptors, but not L-AP4 modulation of ionic currents or inhibitory or excitatory postsynaptic potentials in rat hippocampus. L-AP3 has agonistic activity in enhancing phospholipase D activity and decreasing cAMP formation in rat hippocampus (Schoepp and Conn, 1993). L-AP3 was proposed to have selectivity for metabotropic receptor subtypes based on these differential activities (Schoepp and Conn, 1993). Recently, studies on cloned metabotropic receptors have shown that L-AP3 may have selectivity for Group II subtypes since it inhibits [^{3}H]glutamate binding to $mGlu_3$ receptors (K_i 125 μM) (Laurie et al., 1995) but is inactive in blocking glutamate-stimulated phosphoinositide hydrolysis in Group I subtypes ($mGlu_1$ and $mGlu_5$) (Abe et al., 1992; Aramori and Nakanishi, 1992).

In the present study, a high throughput colorimetic assay for phosphoserine phosphatase is characterized and the discovery that D-and L-AP3 inhibit this enzyme is presented.

2. Materials and methods

2.1. Chemicals

D- and L-phosphoserine were obtained from Sigma and Bachem. Significant color formation with D- and L-phosphoserine obtained from Sigma (presumably due to contaminating inorganic phosphate) was observed at concentrations exceeding 4 and 0.5 mM, respectively. Bachem lots produced much less color at these concentrations and were preferable for saturation experiments. The AP3 enantiomers and other reagents were obtained from Sigma.

2.2. Partial purification of phosphoserine phosphatase

Phosphoserine phosphatase was partially purified according to the method of Bridgers (1967). Rat brain cortices were obtained from ABS (Wilmington, DE). Approx. 22 g cortex was homogenized in ice-cold acetone (~ 3 g cortex/20 ml) using a glass/teflon homogenizer and centrifuged at 6600 × *g* for 20 min at 4°C. The pellets were resuspended in ice-cold acetone (6 g wet weight cortex/10 ml), re-homogenized, and centrifuged at 6600 × *g* for 20 min at 4°C. Acetone powder was produced by drying the pellet under a gentle nitrogen current at 4°C until it was light tan in color and practically free of the smell of acetone. The acetone powder was resuspended and homogenized in Tris-Mg buffer (10 mM Tris-HCl/10 mM $MgCl_2$/1 mM EDTA, pH 7.5) (~ 22 g original wet weight cortex/50 ml), stirred on ice for 30 min, and centrifuged at 18400 × *g* for 35 min. The pellet was resuspended in Tris-Mg buffer (~ 22 g original wet weight cortex/17 ml) and centrifuged at 18400 × *g* for 35 min. The supernatants were combined to produce the acetone powder extract. Ammonium sulfate (13.73 g) was slowly added to 57 ml acetone powder extract on ice and stirred for 30 min. The suspension was centrifuged at 7200 × *g* for 30 min at 4°C. The pellet was discarded and 9.49 g ammonium sulfate was added to 57 ml supernatant and stirred for 1 h on ice. The suspension was centrifuged at 7200 × *g* for 30 min at 4°C. The supernatant was discarded and the pellet was resuspended in 2 ml Tris-Mg buffer and stored at −80°C.

2.3. Phosphoserine phosphatase assay

The thawed, partially purified phosphoserine phosphatase preparation was diluted in 50 mM 2-(*N*-morpho-

lino)ethanesulfonic acid (MES)/5 mM $MgCl_2$, pH 6.2 (MES-Mg buffer) and 150 μl aliquots containing 40–50 μg protein were incubated with 50 μl substrate in MES-Mg buffer for 30 min at 37°C (final volume 250 μl). D- or L-phosphoserine was used as substrate at a concentration of 500 or 100 μM, respectively, in inhibition experiments. Inhibitors were added in 50 μl MES-Mg buffer at the concentrations given in the figures. Increasing concentrations of D- or L-phosphoserine were used in saturation experiments in the absence or presence of 300 μM or 1 mM D-AP3, respectively. The incubations were terminated by addition of 250 μl 1 N HCl and centrifuged on a microfuge at maximal rpm for 10 min. Protein concentration was determined using a modified Lowry procedure (Peterson, 1977).

2.4. Determination of inorganic phosphate

Inorganic phosphate was measured colorimetrically in 96-well polystyrene plates using the method of Fisher and Higgins (1994). Aliquots (100 μl) of the terminated incubation supernatant was mixed with 100 μl malachite green reagent. The malachite green reagent was prepared just prior to use and consisted of 10 ml water, 2 ml 1.3 mM malachite green in 3.6 M H_2SO_4, 0.5 ml 7.5% ammonium molybdate, and 40 μl 11% Tween 20. The standard curve consisted of 0–6 nmol inorganic phosphate added in 100 μl 1 : 1 MES-Mg buffer-1 N HCl. The plate was allowed to stand for 2 h at 22°C and the absorbance at 650 nm determined using a microtiter plate spectrophotometer (Molecular Devices).

2.5. Data analysis

For inhibition experiments, the concentration producing 50% inhibition of enzyme activity (IC_{50}), the maximal extent of inhibition (I_{max}), and the Hill value were estimated using the sigmoidal equation in Prism (GraphPad). The inhibition constant (K_i) was calculated using the Cheng-Prusoff equation (Cheng and Prusoff, 1973). For saturation experiments, the Michaelis constant (K_m) and maximal velocity (V_{max}) were estimated using the hyperbolic equation in Prism.

3. Results

3.1. Standard phosphoserine phosphatase assay

Rat brain cortical phosphoserine phosphatase was partially purified by ammonium sulfate precipitation of an acetone powder extract. This procedure removed components (presumably inorganic phosphate) present in supernatants of rat cortical homogenates which produced high backgrounds in the colorimetric assay. Phosphoserine phosphatase activity was optimal near pH 6.0 as previously

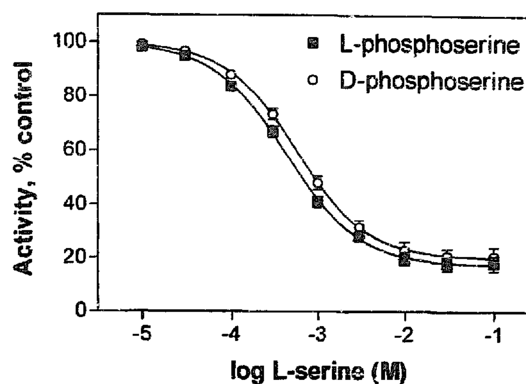

Fig. 3. L-Serine inhibition of rat brain phosphoserine phosphatase. Partially purified enzyme incubated with 500 μM D-phosphoserine (○) or 100 μM L-phosphoserine (■) as substrate for 30 min at 37°C in the presence of increasing concentrations of L-serine. The number of experiments and calculated IC_{50}, I_{max}, and Hill values are shown in Table 1.

described (Bridgers, 1967) and was linear up to 100 μg protein using D-phosphoserine as substrate (not shown). Activity was linear with incubation time up to 45 min at 37°C with 500 μM D-phosphoserine, but was curvilinear with 100 μM L-phosphoserine between 5 and 45 min (not shown). Shorter incubation times or lower concentrations of L-phosphoserine could not be used due to sensitivity limits of the colorimetric assay.

3.2. L-Serine inhibition of phosphoserine phosphatase

L-Serine inhibited phosphoserine phosphatase activity with similar potency and to a similar extent using either D-phosphoserine or L-phosphoserine as substrate (Fig. 3, Table 1). The calculated IC_{50} and I_{max} values are similar to those previously reported (Bridgers, 1967, 1969). The residual phosphatase activity in the presence of saturating L-serine concentrations may be due to sub-maximal inhibition of phosphoserine phosphatase by L-serine or by the presence of contaminating nonspecific phosphatases. The latter possibility is favored since subsequent partial purifications with minor modifications have yielded enzyme

Table 1
Inhibition of rat brain phosphoserine phosphatase by L-serine and AP3

Inhibitor	Substrate	IC_{50} μM	K_i μM	I_{max} %	Hill number
L-Serine	D-Phosphoserine	585 ± 34	–	79 ± 3	1.08 ± 0.03
	L-Phosphoserine	456 ± 15	–	81 ± 4	1.05 ± 0.06
D-AP3	D-Phosphoserine	118 ± 2	48 ± 1	94 ± 8	0.76 ± 0.04
	L-Phosphoserine	988 ± 136	400 ± 55	96 ± 2	0.82 ± 0.02
DL-AP3	D-Phosphoserine	187 ± 11	77 ± 5	94 ± 3	0.75 ± 0.01
	L-Phosphoserine	1403 ± 57	568 ± 23	101 ± 1	0.82 ± 0.02
L-AP3	D-Phosphoserine	368 ± 27	151 ± 11	98 ± 4	0.73 ± 0.03
	L-Phosphoserine	2087 ± 92	845 ± 37	104 ± 1	0.86 ± 0.04

Partially purified enzyme incubated with 500 μM D-phosphoserine or 100 μM L-phosphoserine as substrate for 30 min at 37°C in the presence of increasing concentrations of inhibitor in duplicate. Values are means ± S.E.M. of three (D-, L-, DL-AP3) or four (L-serine) independent experiments.

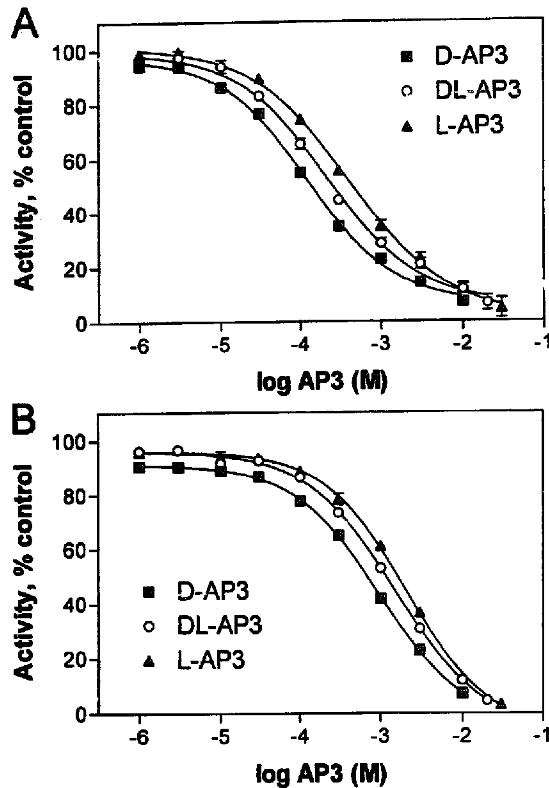

Fig. 4. Inhibition of rat brain phosphoserine phosphatase by AP3 enantiomers. Partially purified enzyme incubated with substrate for 30 min at 37°C in the presence of increasing concentrations of D-AP3 (■), DL-AP3 (○), or L-AP3 (▲). (A) D-Phosphoserine (500 μM) as substrate. (B) L-Phosphoserine (100 μM) as substrate. The number of experiments and calculated IC_{50}, I_{max}, and Hill values are shown in Table 1.

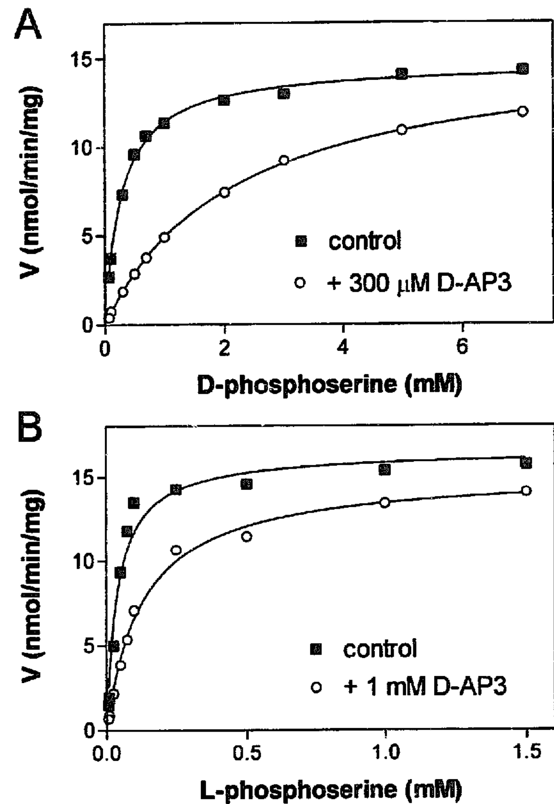

Fig. 5. Saturation of rat brain phosphoserine phosphatase in the presence and absence of D-AP3. Partially purified enzyme incubated with increasing concentrations of substrate for 30 min at 37°C in the absence (■) or presence of D-AP3 (○). (A) D-Phosphoserine as substrate. For this representative experiment, K_m and V_{max} values were 288 μM and 15 nmol/min/mg in the absence of D-AP3 and 2178 μM and 16 nmol/min/mg in the presence of 300 μM D-AP3. (B) L-Phosphoserine as substrate. For this representative experiment, K_m and V_{max} values were 41 μM and 16 nmol/min/mg in the absence of D-AP3 and 130 μM and 15 nmol/min/mg in the presence of 1 mM D-AP3. The overall K_m and V_{max} values from replicate experiments are shown in Table 2.

preparations with virtually complete inhibition of activity at saturating L-serine concentrations (not shown). Control experiments indicate that L-serine neither generates color nor interferes with color formation produced by inorganic phosphate.

3.3. AP3 inhibition of phosphoserine phosphatase

AP3 completely inhibits phosphoserine phosphatase activity (Fig. 4, Table 1). The D-isomer of AP3 is 2- to 3-fold more potent than the L-isomer as an inhibitor of phosphoserine phosphatase depending on substrate, whereas the racemic mixture is intermediate in potency. D-AP3 potently inhibits phosphoserine phosphatase with a K_i of 48 μM using D-phosphoserine as substrate (Fig. 4A, Table 1). Interestingly, D-AP3 is 8.4-fold less potent using L-phosphoserine as substrate (Fig. 4B, Table 1). This substrate-dependent potency difference is less apparent with L-AP3, which is a 5.7-fold more potent inhibitor of phosphoserine phosphatase using D-phosphoserine compared to L-phosphoserine as substrate. The Hill slopes of the concentration-inhibition curves were consistently < 1.0. Control experiments indicate that neither D- nor L-AP3 is a substrate and neither inhibitor generates color nor interferes with color formation produced by inorganic phosphate.

3.4. Saturation analysis of phosphoserine phosphatase

Saturation experiments indicate that L-phosphoserine has higher affinity (K_m 68 μM) for phosphoserine phosphatase than D-phosphoserine (K_m 347 μM) although the enzyme cleaves these substrates with similar maximal

Table 2
Saturation of rat brain phosphoserine phosphatase in the presence and absence of D-AP3

Substrate	D-AP3	K_m μM	V_{max} nmol/min/mg
D-Phosphoserine	–	347 ± 52	16 ± 1
	300 μM	1885 ± 272	16 ± 1
L-Phosphoserine	–	68 ± 11	18 ± 1
	1 mM	160 ± 28	16 ± 1

Partially purified enzyme incubated with increasing concentrations of L-phosphoserine or D-phosphoserine as substrate in duplicate for 30 min at 37°C in the presence or absence of D-AP3. Values are means ± S.E.M. of four (D-AP3) or eight (control) independent experiments.

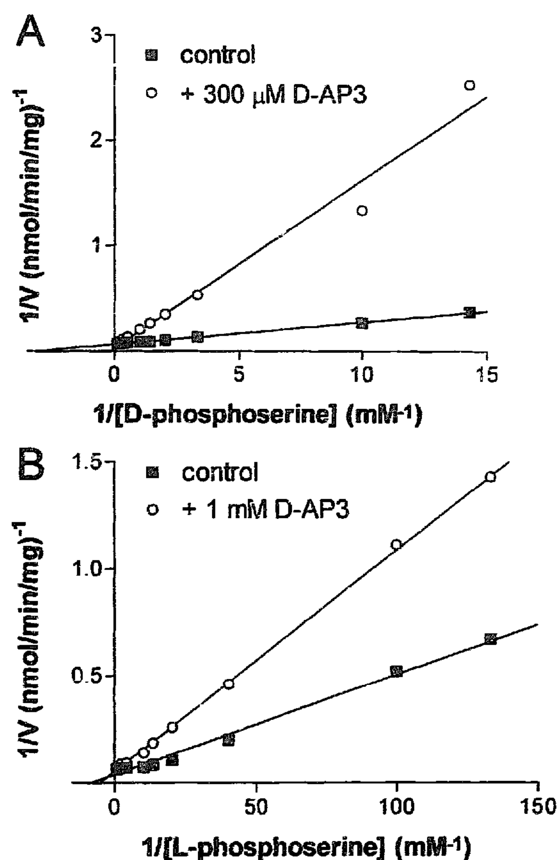

Fig. 6. Lineweaver-Burke transformation of the saturation data in Fig. 5. (A) D-Phosphoserine as substrate. (B) L-Phosphoserine as substrate.

velocities (Fig. 5A,B, Table 2). These K_m values for rat cortex are 2- to 3-fold higher than previously reported in mouse and human brain (Bridgers, 1967; Veeranna and Shetty, 1991). Saturation experiments indicate that D-AP3 is a competitive inhibitor of phosphoserine phosphatase since the apparent K_m is higher in the presence of D-AP3 with little or no change in V_{max} using both D- and L-phosphoserine as substrates (Fig. 5A,B, Table 2). The competitive inhibition of phosphoserine phosphatase by D-AP3 is apparent in Lineweaver-Burke transformations of the saturation data (Fig. 6A,B).

4. Discussion

Phosphoserine phosphatase is a key enzyme regulating the biosynthesis of L-serine in brain. In addition to its role in protein synthesis, L-serine is the precursor of complex lipids (e.g., sphingomyelins and gangliosides) and also glycine via the serine hydroxymethyl transferase reaction. Indeed, the glycine concentration in rat brain during development is correlated with phosphoserine phosphatase activity (McChesney et al., 1987). Like L-serine, glycine has multiple roles in addition to protein synthesis. Glycine is a component of glutathione, is involved in purine biosynthesis, and functions as a neurotransmitter. Glycine is the endogenous agonist at strychnine-sensitive glycine receptors, where it gates a chloride channel-mediating inhibitory transmission, particularly in the spinal cord (Bechade et al., 1994). Glycine also functions as an obligatory coagonist at strychnine-insensitive glycine sites associated with the NMDA subtype of excitatory amino acid receptors (Wood, 1995).

In contrast to rat brain where it is uniformly distributed, phosphoserine phosphatase is differentially distributed in human brain regions. Phosphoserine phosphatase activity is enriched in human hippocampus compared to cortex, whereas other regions have intermediate levels (Veeranna and Shetty, 1991). Although complicated by the diverse roles of L-serine and glycine in brain metabolism, the differential distribution of human phosphoserine phosphatase may be related in part to regional requirements for the neurotransmitter functions of glycine. Although little is known about the subcellular distribution of phosphoserine phosphatase, rat cortical synaptosomes cleave L-phosphoserine suggesting that high levels of this enzyme exist presynaptically (Wood et al., 1995).

A novel microtiter plate assay for phosphoserine phosphatase was characterized which measures inorganic phosphate production colorimetrically. Phosphoserine phosphatase was partially purified from rat cortex, which reduced interference from color forming contaminants, presumably inorganic phosphate. Phosphoserine phosphatase activity was characterized using both D- and L-phosphoserine as substrates. L-Phosphoserine is the natural substrate for the enzyme and has higher affinity than D-phosphoserine, but gives non-linear kinetics possibly due to end product inhibition by L-serine. Although D-phosphoserine has lower affinity for phosphoserine phosphatase, it gives linear kinetics. Since it is not detectable in rat brain (Goodnough et al., 1995), D-phosphoserine cannot be a natural substrate of phosphoserine phosphatase, consistent with its relatively high K_m and the lack of inhibition by the end product D-serine ($IC_{50} > 10$ mM) (Grillo and Cogue, 1966).

L-Serine is a noncompetitive or uncompetitive end product inhibitor of phosphoserine phosphatase (Bridgers, 1967; Veeranna and Shetty, 1991) and is the only known micromolar inhibitor of this enzyme. The potency of L-serine for inhibition of phosphoserine phosphatase is similar using D-phosphoserine or L-phosphoserine as substrate indicating that both substrates give accurate estimates of the potency of this allosteric inhibitor and that L-serine inhibits all isozymes of phosphoserine phosphatase that may be present. The previously reported inhibitors trifluoperazine and chloropromazine are quite weak since < 50% inhibition was observed at 1 mM (Veeranna and Shetty, 1991). The only other known inhibitor is the sulfhydryl reagent N-ethylmaleimide (Paoli et al., 1974).

The striking structural similarities between AP4 and phosphoserine (Fig. 2) suggested that AP4 might be a potent competitive inhibitor of phosphoserine phosphatase.

Indeed, this structural similarity has previously been exploited in the metabotropic receptor area since L-phosphoserine is known to be an agonist at L-AP4 receptors ($mGlu_{4-8}$) (Knopfel et al., 1995). However, DL-AP4 (500 μM) was found to be inactive as an inhibitor of phosphoserine phosphatase (J.E. Hawkinson, M. Acosta-Burruel and P. Wood, unpublished observations). Surprisingly, D-AP3 (Fig. 2), an analog of D-AP4 containing one less methylene carbon, was found to be a potent inhibitor of phosphoserine phosphatase. D-AP3 is not a substrate since it possesses a non-cleavable phosphonate group rather than the phosphoryl group present in all known substrates. The inhibition by AP3 is stereospecific since the D-enantiomer is more potent than the L-enantiomer whereas racemic DL-AP3 has intermediate potency.

Both D- and L-AP3 are more potent using D-phosphoserine as substrate relative to L-phosphoserine, suggesting that multiple phosphoserine phosphatase isozymes may be present in the enzyme preparation and that D- and L-AP3 have higher affinity for isoforms which preferentially cleave D-phosphoserine. This conclusion is supported by the low Hill values for D- and L-AP3 concentration-inhibition curves. Indeed, several isoforms of phosphoserine phosphatase have been identified in human brain (Moro-Furlani et al., 1980). Saturation experiments indicate that D-AP3 is a competitive inhibitor of phosphoserine phosphatase consistent with its structural similarity to the natural substrate. In view of its competitive interaction at the active site, it is surprising that AP3 displays the opposite enantioselectivity (D-AP3 affinity > L-AP3) relative to the substrate (L-phosphoserine affinity > D-phosphoserine). Although D-AP3 is competitive with both substrates, D- and L-phosphoserine may bind to different domains within the active site with different degrees of overlap by D-AP3 which could explain the difference in potency of this inhibitor with the two different substrates. Resolution of these issues awaits inhibition studies using pure isoforms and/or crystallographic elucidation of the structure of the active site.

In contrast to its enantioselectivity at phosphoserine phosphatase, the L-isomer of AP3 is a more potent antagonist than the D-isomer at metabotropic glutamate receptors. For example, L-AP3 is 3- or 5-fold more potent than D-AP3 as an inhibitor of ibotenate- or quisqualate-stimulated phosphoinositide hydrolysis in hippocampal or cortical slices, respectively (Schoepp et al., 1990). AP3 blocks ibotenate-stimulated phosphoinositide hydrolysis with IC_{50} values of 168 and 515 μM for the L- and D-isomers, respectively (Schoepp et al., 1990). Thus, L-AP3 is approximately equipotent as an inhibitor of phosphoserine phosphatase (K_i 151 μM using D-phosphoserine as substrate) and metabotropic receptors, whereas D-AP3 is a 10-fold more potent inhibitor of phosphoserine phosphatase (K_i 48 μM using D-phosphoserine as substrate) than metabotropic receptors. Interestingly, L-AP3 is thought to be a noncompetitive inhibitor of metabotropic receptors (Schoepp et al., 1990), although competitive inhibition has also been reported (Saugstad et al., 1995). D-AP3 has also been reported to bind the NMDA receptor (Schoepp et al., 1990), but it has higher affinity for phosphoserine phosphatase than this subtype of ionotropic receptor ([^{3}H]CGS 19755 binding IC_{50} 292 μM).

The inhibition of metabotropic glutamate receptors by L-AP3 has been questioned since it inhibits phosphoinositide synthesis from [^{3}H]*myo*-inositol in hippocampal slices under the same conditions in which it inhibits metabotropic agonist-induced phosphoinositide hydrolysis (Ikeda, 1993). In the present study, the inhibition of phosphoserine phosphatase by L-AP3 provides further evidence for multiple actions of L-AP3 and complicates the interpretation of essentially all functional data obtained with L-AP3 where inhibition of serine and glycine biosynthesis can affect the result. In contrast, D-AP3 is a selective phosphoserine phosphatase inhibitor and inhibition of this enzyme must be considered when interpreting functional data obtained with D-AP3 or DL-AP3.

In vivo, D- or L-AP3 must cross cellular membranes to inhibit the cytosolic enzyme phosphoserine phosphatase, whereas inhibition of membrane-bound metabotropic glutamate receptors presumably does not require membrane permeation. In this regard, L-AP3 has been shown to be a potent inhibitor of sodium-dependent L-[^{3}H]glutamate uptake in cerebellar and cortical synaptosomes (39 and 110 μM, respectively) (Robinson et al., 1993), suggesting that it may be a substrate of the glutamate transporter. In support of this possibility, L-AP3 produces an inward sodium current in cultured hippocampal astrocytes consistent with uptake via the glutamate transporter (Kirby-Sharkey et al., 1995). These results suggest that L-AP3 gains access to the intracellular compartment where it can inhibit phosphoserine phosphatase. To our knowledge, the interaction of D-AP3 with glutamate transporters has not been evaluated.

L-AP3 has anticonvulsant activity in animals. For example, L-AP3 blocks audiogenic seizures in DBA/2 mice (Klitgaard and Jackson, 1993). In addition, DL-AP3 has been shown to be neuroprotective in the gerbil model of global ischemia (Maginn et al., 1995). It is possible that these anticonvulsant and neuroprotective activities may be related to inhibition of phosphoserine phosphatase with subsequent reductions in brain glycine availability. Although it has poor bioavailability, D-AP3 is the best available pharmacologic tool to explore these possibilities since it is noncleavable and is the most potent and selective known inhibitor of this enzyme.

References

Abe, T., H. Sugihara, H. Nawa, R. Shigemoto, N. Mizuno and S. Nakanishi, 1992, Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/Ca^{2+} signal transduction, J. Biol. Chem. 267, 13361.

Aramori, I. and S. Nakanishi, 1992, Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells, Neuron 8, 757.

Bechade, C., C. Sur and A. Triller, 1994, The inhibitory neuronal glycine receptor, BioEssays 16, 735.

Bridgers, W.F., 1965, The biosynthesis of serine in mouse brain extracts, J. Biol. Chem. 240, 4591.

Bridgers, W.F., 1967, Mouse brain phosphoserine phosphohydrolase and phosphotransferase, J. Biol. Chem. 242, 2080.

Bridgers, W.F., 1969, Purification of mouse brain phosphoserine phosphohydrolase and phosphotransferase, Arch. Biochem. Biophys. 133, 201.

Cheng, Y.-C. and W.H. Prusoff, 1973, Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzymatic reaction, Biochem. Pharmacol. 22, 3099.

Fisher, D.K. and T.J. Higgins, 1994, A sensitive, high-volume, colorimetric assay for protein phosphatases, Pharmacol. Res. 11, 759.

Goodnough, D.B., M.P. Lutz and P.L. Wood, 1995, Separation and quantification of D- and L-phosphoserine in rat brain using *Nα*-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (Marfey's reagent) by high-performance liquid chromatography with ultraviolet detection, J. Chromatogr. B 672, 290.

Grillo, M.A. and M. Cogue, 1966, Phosphoserine phosphatase of vertebrates, Comp. Biochem. Physiol. 18, 169.

Ikeda, M., 1993, Reduction of phosphoinositide hydrolysis by L-amino-3-phosphonopropionate may be caused by the inhibition of synthesis of phosphatidylinositols, Neurosci. Lett. 157, 87.

Kirby-Sharkey, C.O., Y. Izumi, S. Mennerick, A.M. Benz, J. Labruyere, C.F. Zorumski, M.T. Price and J.W. Olney, 1995, Glial swelling by AP3 and glutamate transport inhibitors in the retina, Soc. Neurosci. Abstr. 21, 1861.

Klitgaard, H. and H.C. Jackson, 1993, Central administration of L-amino-3-phosphoropropionate (L-AP3) inhibits audiogenic seizures in DBA/2 mice, Funct. Neurol. 8 (Suppl. 4), 28.

Knopfel, T., R. Kuhn and H. Allgeier, 1995, Metabotropic glutamate receptors: novel targets for drug development, J. Med. Chem. 38, 1417.

Laurie, D.J., M. Danzeisen, H.W.G.M. Boddeke and B. Sommer, 1995, Ligand binding profile of the rat metabotropic glutamate receptor mGluR3 expressed in a transfected cell line, Naunyn-Schmied. Arch. Pharmacol. 351, 565.

Maginn, M., M. Caldwell, J.P. Kelly and B.E. Leonard, 1995, The effect of 2-amino-3-phosphonopropionic acid (AP-3) in the gerbil model of cerebral ischemia, Eur. J. Pharmacol. 282, 259.

McChesney, R., C.E. Isaacs and O. Greengard, 1987, Cerebral glycine content and phosphoserine phosphatase activity in hyperacidemias, Neurochem. Res. 12, 289.

Moro-Furlani, A.M., V.S. Turner and D.A. Hopkinson, 1980, Genetical and biochemical studies on human phosphoserine phosphatase, Ann. Hum. Gen. London 43, 323.

Neuwald, A.F. and G.V. Stauffer, 1985, DNA sequence and characterization of the *Escherichia coli serB* gene, Nucl. Acids Res. 13, 7025.

Paoli, A., P. Guiraud and C. Brunel, 1974, *O*-Phosphoserine phosphatase from bovine brain and kidney: High molecular weight forms occurring during purification, Biochim. Biophys. Acta 370, 487.

Peterson, G.L., 1977, A simplification of the protein assay method of Lowry et al. which is more generally applicable, Anal. Biochem. 83, 346.

Robinson, M.B., J.D. Sinoor, L.A. Dowd and J.F. Kerwin, 1993, Subtypes of sodium-dependent high-affinity L-[^{3}H]glutamate transport activity: pharmacologic specificity and regulation by sodium and potassium, J. Neurochem. 60, 167.

Saugstad, J.A., T.P. Segerson and G.L. Westbrook, 1995, L-2-Amino-3-phosphonopropionic acid competitively antagonizes metabotropic glutamate receptors 1α and 5 in *Xenopus* oocytes, Eur. J. Pharmacol. 289, 395.

Schoepp, D.D. and P.J. Conn, 1993, Metabotropic glutamate receptors in brain function and pathology, Trends Pharmacol. Sci. 14, 13.

Schoepp, D.D. and B.G. Johnson, 1989, Comparison of the amino acid-stimulated phosphoinositide hydrolysis and *N*-[^{3}H]acetylaspartylglutamate binding in rat brain: selective inhibition of phosphoinositide hydrolysis by 2-amino-3-phosphonopropionate, J. Neurochem. 53, 273.

Schoepp, D.D., B.G. Johnson, E.C.R. Smith and L.A. McQuaid, 1990, Stereoselectivity and mode of inhibition of phosphoinositide-coupled excitatory amino acid receptors by 2-amino-3-phosphonopropionic acid, Mol. Pharmacol. 38, 222.

Veeranna and K.T. Shetty, 1991, Phosphoserine phosphatase of human brain: partial purification, characterization, regional distribution, and effect of certain modulators including psychoactive drugs, Neurochem. Res. 16, 1203.

Wood, P.L., 1995, The co-agonist concept: Is the NMDA-associated glycine receptor saturated in vivo?, Life Sci. 57, 301.

Wood, P.L., D.B. Goodnough, J.E. Hawkinson and R.P. Shank, 1995, Presynaptic synthesis of the NMDA-associated glycine receptor agonist, D-serine, Soc. Neurosci. Abstr. 21, 1265.